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THE EFFECT OF X-RADIATION UPON SOMATIC CHROMOSOMES.

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(With Plates I-II and Thirty-three Text-figures.)

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I. INTRODUCTION.

THE use of X-radiation as a means of causing rearrangement of the genetic materials of an organism has led to very important results, particularly in *Drosophila melanogaster* and *Zea Mays*. The resulting organisms have been examined to some extent cytologically and analysed genetically, and only certain types of chromosome rearrangement, viz. translocations and deletions, have been found, but no attempt has been made to find out why these abnormalities and these alone should occur. The following observations provide a cytological basis for the observed genetical results, and also provide some evidence on the still very open question of the evolution of chromosome complements.

II. MATERIAL AND METHODS.

Crocus Olivieri ($2n = 6$), *Crocus Balansae* ($2n = 6$) and *Crocus biflorus* ($2n = 8$) were chosen as material for this investigation on account of their low chromosome number. Some plants of *Tulipa Gesneriana*

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(variety Philippe de Commynes), which had been irradiated for other purposes, were also examined to confirm the observations on *Crocus*.

The X-ray apparatus consists of a Coolidge type water-cooled tube, and all irradiations were unscreened.

Two treatments of *Crocus Olivieri* were made. In each case the intensity of the radiation was constant but the length of the exposure varied. The treatments were as follows:

(1) 64 kilovolts, 5 milliamps., 30 cm. target distance: 15 min. unscreened irradiation.

(2) 64 kilovolts, 5 milliamps., 30 cm. target distance: 30 min. unscreened irradiation.

The well-rooted corms were treated when in pans of fibre, and were then lifted, carefully washed, and placed in a Petri dish containing water. Root tips from both corms were fixed at the following intervals¹:

(1) 30 min. after treatment

(2) 7 hr. ,,

(3) 24 hr. ,,

(4) 48 hr. ,,

(5) 72 hr. ,,

The corms were then potted up and root tips were again fixed after a little over three months had elapsed.

C. Balansae and *C. biflorus* were treated in one way only. Corms of both species were grown in fibre until well rooted and were then lifted, washed well and irradiated as in the first treatment of *C. Olivieri*, viz.

64 kv., 5 ma., 30 cm., 15 min. unscreened radiation.

The corms were then placed with their roots dipping into Knop's nutrient solution. Root tips from each species were fixed:

(1) 2 days after treatment

(2) 3 ,,

(3) 4 ,,

(4) 5 ,,

(5) 8 ,,

(6) 14 ,,

The corms were then potted up and one more fixation was made 35 days after irradiation.

¹ This material was previously used by Stone (1933) for a determination of the time of occurrence of abnormalities.

Tulips. Five different samples of resting bulbs were treated on

- (1) June 28
- (2) July 11
- (3) Aug. 17
- (4) Sept. 19
- (5) Oct. 25

in each case at 90 kv. 5 ma. at a target distance of 30 cm. for (a) 3 min., (b) 5 min., (c) 7 min. Bulbs¹ from treatments 1-4 were potted in October and root tips were fixed in late December. Bulbs from treatment 5 were potted in November and root tips were fixed in early February; at the same time more roots of treatment 4 were fixed.

In all cases root tips were fixed in La Cour's 2 BE. *Crocus* roots were sectioned at 20μ and *Tulipa* roots at 28μ . The slides were stained by Newton's gentian-violet-iodine method.

Fixation was slightly variable in *Crocus* but was on the whole very good. All fixation in *Tulipa* was good.

III. OBSERVATIONS.

(i) *Crocus*.

Stone (1933) has shown that the behaviour of chromosomes in root tips of *C. Olivieri* exhibits three phases after irradiation. First of all any mitoses which are already in progress during irradiation are completed without the occurrence of any abnormalities. Then ensues a long resting stage, lasting 2 days after the heavier dosages, and during this period no signs of mitosis are to be seen. Finally divisions begin again and the chromosomes show abnormalities. It is with this last phase that the present observations are concerned.

The normal chromosomes of the three species of *Crocus* are illustrated in Text-figs. 1-3. Those of *C. Olivieri* and *C. Balansae* are rather similar, consisting of three pairs all having a sub-terminal attachment constriction. The pair referred to in this paper as *A* also have a long secondary constriction proximal to the attachment and separated from it by a very small knob of chromatin. Pairs *B* and *C* have no secondary constrictions but may be distinguished from each other by the length of their proximal arms.

C. biflorus has four pairs of chromosomes, and the pairs are not so easily distinguished as in the other two species. Two pairs have median or sub-median attachment constrictions (*M*), and two have sub-terminal

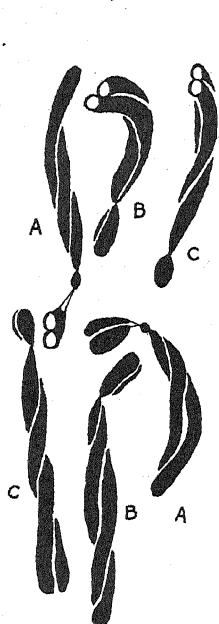
¹ Meiosis in sister bulbs was examined by Stone (1933).

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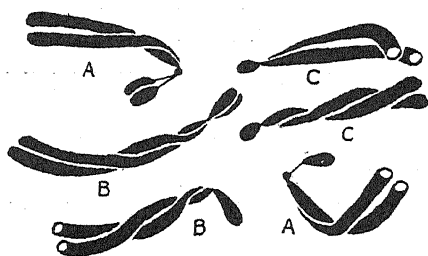
ones (*T*). One of the latter pairs also has rather large trabants on the proximal arms.

In all three species the first abnormal metaphases are seen in the root tips fixed 2 days after irradiation, but they are few. In root tips fixed 3 or more days after irradiation they are common.

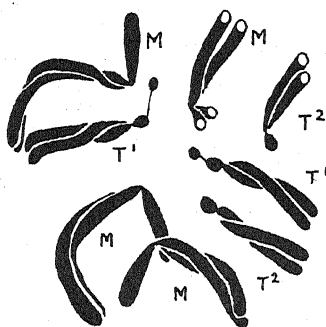
Abnormalities are very marked in roots fixed 3 days after treatment. Often as many as 10 chromosome bodies are to be seen in the cell. In no



Text-fig. 1.



Text-fig. 2.



Text-fig. 3.

Text-figs. 1-3. The normal somatic chromosomes of Fig. 1, *C. Olivieri*; Fig. 2, *C. Balansae*; Fig. 3, *C. biflorus*. $\times 4000$.

case are they all orientated completely on the metaphase plate, indeed some of the bodies lie at random throughout the cell. A certain number of them do, however, orientate themselves at metaphase precisely as chromosomes do in normal mitosis. Obviously these two classes of chromosome bodies differ in some very definite and clear-cut manner. That this orientation or non-orientation is not a function of the length of the chromosome is shown by the fact that both long and short chromosomes lie on the metaphase plate and similarly both long and short ones lie off it (Text-fig. 14).

There is, however, one characteristic of the number of bodies which do orientate themselves on the plate, viz. in the case of *C. Olivieri* and

C. Balansae they almost always total 6, and in *C. biflorus* they almost always total 8, i.e. in each case the number of chromosome bodies which do possess the property of orientation between the poles at metaphase is equal to the number of somatic chromosomes present before abnormalities were induced. A careful comparative examination showed that these 6 (or in *C. biflorus* 8) chromosomes possess an attachment constriction, and this apparently endows them with the property of normal orientation on the equatorial plate at metaphase, while the other bodies do not possess attachment constrictions and consequently are not chromosomes in the strict sense, as they do not behave in the normal manner.

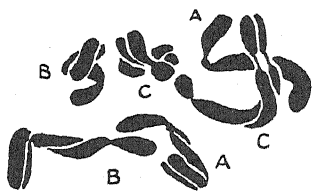
These two types of chromosome or chromosome-like bodies will be referred to as proximal and distal fragments according as they do or do not respectively possess points of attachment, following the nomenclature of Navashin (1931). Text-figs. 5, 13, 14, 21, 22 illustrate mitotic metaphases showing the two types of bodies.

The behaviour of proximal and distal fragments at anaphase is typical. The proximal ones possessing an attachment constriction separate to the two poles like normal chromosomes. The distal fragments, possessing no points of attachment, fail to separate and can be seen as double bodies lying at various places in the cell with no orientation at all (see Text-figs. 16, 29, and Plate I, figs. 1-6). Thus they must, in the majority of cases, fail to get included in the daughter nuclei. That length has nothing to do with this power of separation is shown by Text-fig. 29, where a very short chromosome with a point of attachment is separating to the poles, while two long fragments without attachments are not.

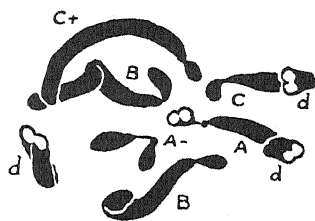
The behaviour of these distal fragments offers an interesting comparison with the behaviour of univalents at meiosis. In both cases the normal mechanism of separation is lacking and so both lag on the equator. The univalent, however, may divide at anaphase and so recover the normal separation mechanism, leading to disjunction of the two halves, but the distal fragment can never develop a mechanism of separation and so always lags.

In root tips fixed on the fourth day after treatment, some mitoses still show both proximal and distal fragments, but a number of divisions are present showing nothing but proximal fragments behaving in the normal manner.

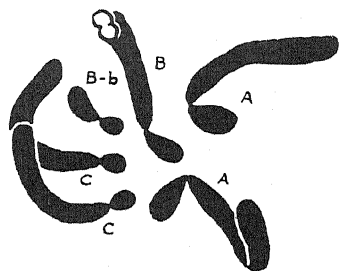
In root tips fixed on the fifth day after treatment, distal fragments are exceedingly rare and where present usually show signs of disintegration. In tips fixed more than 5 days after irradiation nothing but proximal fragments are to be seen.



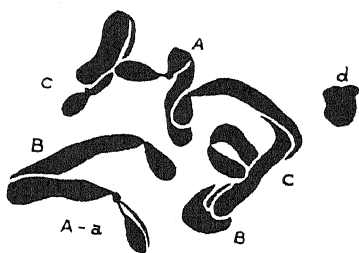
Text-fig. 4.



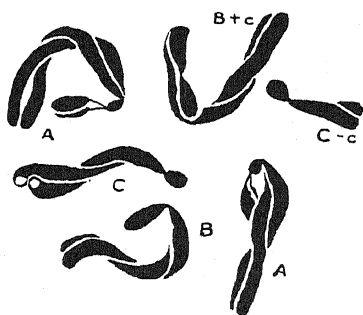
Text-fig. 5.



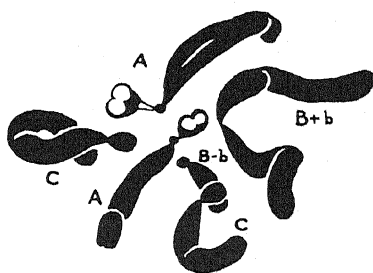
Text-fig. 6.



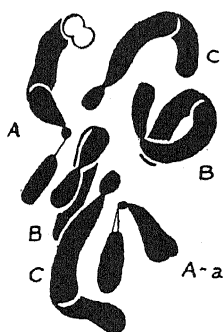
Text-fig. 7.



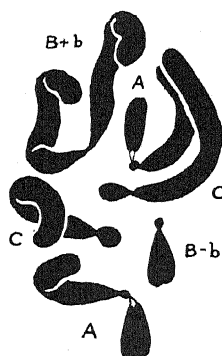
Text-fig. 8.



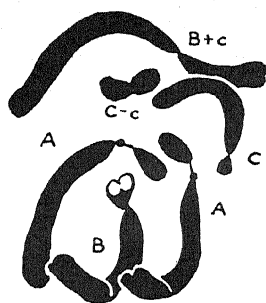
Text-fig. 9.



Text-fig. 10.

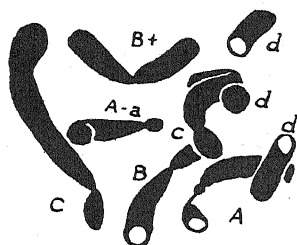


Text-fig. 11.

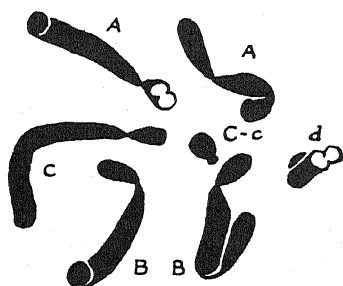


Text-fig. 12.

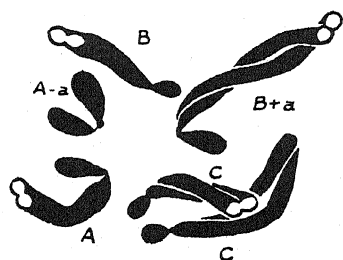
Text-figs. 4-12. Somatic metaphases from irradiated root tips of *C. Olivieri*. Figs. 4 and 5, two days after treatment. Figs. 6-8 and 12, three days after treatment. Figs. 9-11, three months after treatment. Fig. 5 shows distal fragments (*d*). Fig. 7 shows a degenerating distal fragment. Figs. 9 and 11 show the same abnormality and are from the same sector of the root tip, which is therefore a chimaera for this abnormality. $\times 4000$.



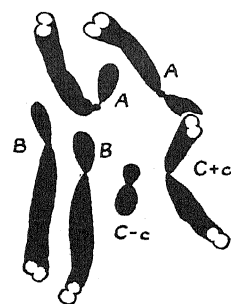
Text-fig. 13.



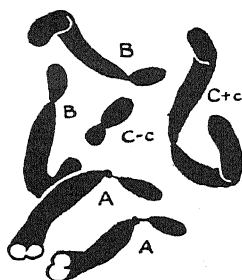
Text-fig. 14.



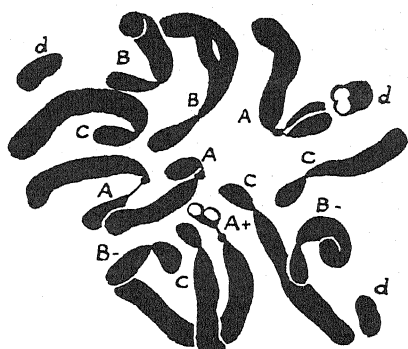
Text-fig. 17.



Text-fig. 19.



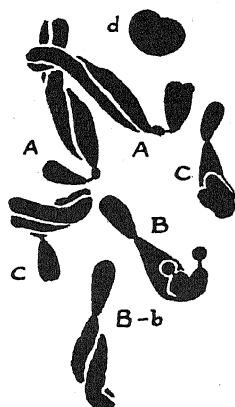
Text-fig. 20.



Text-fig. 15.



Text-fig. 16.



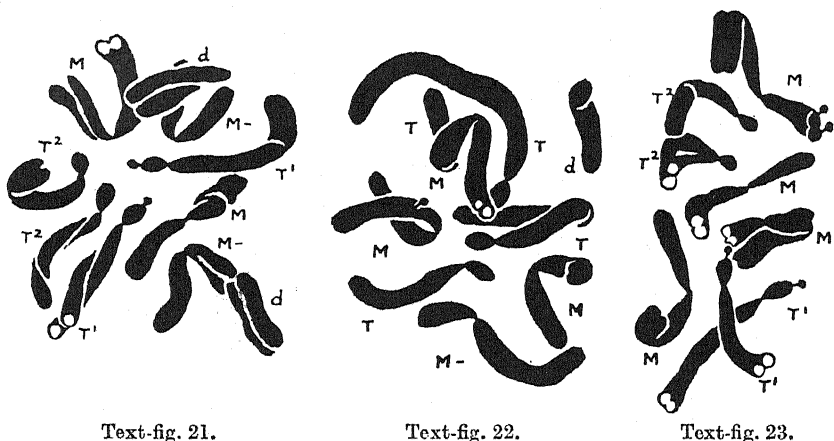
Text-fig. 18.

Text-figs. 13-20. Somatic metaphases, except Fig. 16 which is anaphase from irradiated root tips of *C. Balansae*. Fig. 13, three days; Fig. 14, four days; Figs. 15-18, five days; Figs. 19, 20, thirty-five days after treatment. Figs. 13, 14 and 18 show distal fragments (*d*) (degenerating in 18) at metaphase. Fig. 16 illustrates their behaviour at anaphase—note how they lie off the plate and fail to separate (cf. Plate I, figs. 1-6). Fig. 14 shows a very small proximal fragment orientating itself and a large distal fragment not orientating itself at metaphase. Fig. 15 is a tetraploid cell (from a diploid root) showing distal fragments. The cell was tetraploid before irradiation. Figs. 19 and 20 are from the same sector of a root tip and show the same abnormality (cf. Figs. 9 and 11). $\times 4000$.

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Hence unless a chromosome, or more precisely chromosome body, possesses an attachment constriction it cannot become part of the permanent hereditary complement of the nucleus.

It does not follow that all the distal fragments are lost. Many of them fuse with a proximal fragment, and so persist as translocations. This is shown by almost every mitotic metaphase examined. Several examples are illustrated in the various text-figures. In some cells the origin of the translocations can be made out. This is indicated in the text-figures wherever it could be ascertained. A capital letter indicates the original



Text-figs. 21-23. Somatic metaphases from irradiated root tips of *C. biflorus*. Figs. 21 and 22, four days after treatment. Fig. 23, five days after treatment. Note the presence of lateral trabants in Figs. 22 and 23, and distal fragments (*d*) in Figs. 21 and 22. $\times 4000$.

chromosome and a small letter the distal fragment involved in the translocation. In many cases, however, it can be seen that one chromosome has had a piece broken off, but no other chromosome appears to be long enough to account for the subsequent fusion of the resulting distal fragment. In such cases either of two things may have happened: (1) the distal fragment may have been lost, owing to its not fusing with a proximal fragment or unaffected chromosome, or (2) it may have become further subdivided and each piece joined on to a different proximal segment or unaffected chromosome. In the former case the genic balance of the nucleus would be upset and might result in the cessation of division or even death of the nucleus and cell. The latter case would not affect the balance of the nucleus unless a "position" effect came into action, such as has been shown genetically to be the case in certain translocations in

Drosophila. It needs more delicate methods than direct cytological observation to determine if such is the case in *Crocus*.

It will be seen that briefly the course taken by the abnormal nuclei is as follows:

(i) Abnormalities arise consisting in the formation of proximal and distal fragments by breaking of the chromosomes, and distal fragments may or may not fuse with another chromosome body.

(ii) The abnormal nucleus passes into division and only the unaffected chromosomes and proximal fragments orient themselves normally between the poles at metaphase.

(iii) At anaphase and telophase the unattached distal fragments apparently lag and get lost.

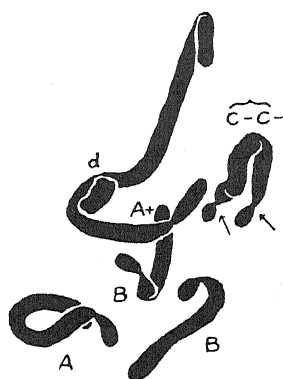
(iv) In all subsequent cell divisions no unattached distal fragments are present. However, the chromosomes may and usually do show translocations.

Where a nucleus has become abnormal by the occurrence of one or more translocations but is otherwise unaffected as to genetic balance, it will divide normally and eventually the root tip containing it will have a whole sector showing the abnormality. Examples of root tips chimerical for an abnormality in this way have been found in fixations several weeks after irradiation. Text-figs. 9 and 11 show two divisions from the same sector of a root tip of *C. Olivieri* in which one *B*-chromosome has been broken and the distal fragment joined on to the short arm of the other *B*-chromosome. Text-figs. 19 and 20 illustrate the same phenomenon in *C. Balansae*, the *C*-chromosomes being involved here.

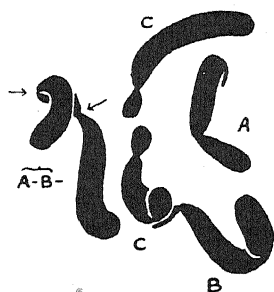
A few mitoses in all three species, particularly in the earlier fixations, show chromosomes behaving in a rather peculiar manner. In these nuclei one less than the normal somatic number of chromosomes appear on the metaphase plate, but in each case one chromosome has an unusual appearance. These chromosomes appear to possess two attachment constrictions. At metaphase they show two definite constrictions, and the orientation at these two points is that which is characteristic of the points of attachment. The constrictions themselves are orientated on the equatorial plate while the rest of the chromosome is directed away from the centre of the plate and in many cases slightly off it. This is the typical behaviour of points of attachment and the rest of the chromosome in normal cells (cf. Text-figs. 24-28 and Plate I, figs. 7-12).

The critical evidence as to the precise nature of these chromosomes can only be derived from their anaphase separation. An intensive search was made for anaphases showing such chromosomes dividing and two were

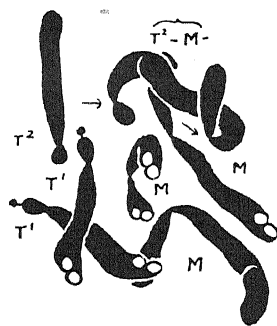
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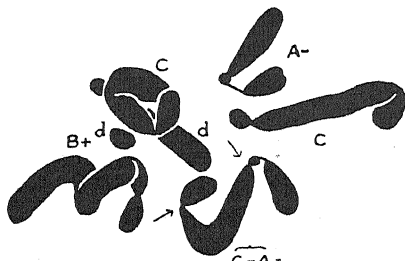
Text-fig. 24.



Text-fig. 26.



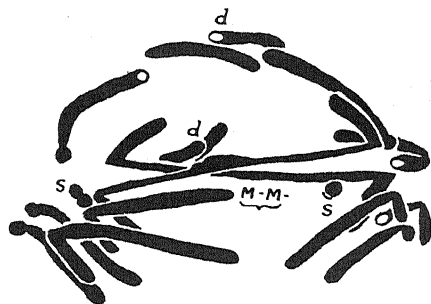
Text-fig. 28.



Text-fig. 25.



Text-fig. 27.



Text-fig. 29.

Text-figs. 24-28. Somatic metaphases showing chromosomes with two points of attachment and Fig. 29, somatic anaphase showing the separation of such a chromosome. Fig. 24, *C. Olivieri* two days after treatment. Figs. 25 and 26, *C. Balansae* three and four days after treatment respectively. Figs. 27-29, *C. biflorus*. Figs. 27 and 29, four days and Fig. 28, five days after treatment. Arrows indicate the points of attachment in chromosomes with two of them.

Note the orientation of the chromosomes at metaphase and how they become drawn out at anaphase. In Fig. 29 also note the lagging and failure to divide of the long distal fragments (*d*) and the separation of the very short proximal fragment (*s*) (cf. Plate II, figs. 13-19). $\times 4000$.

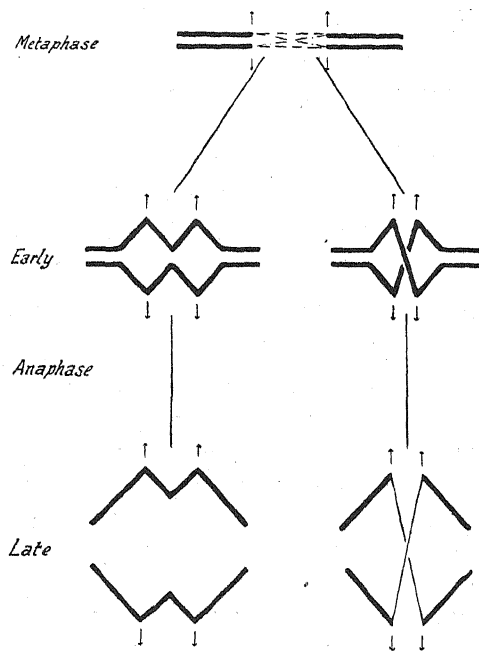
found in *C. biflorus*. One was a side view and is shown in Text-fig. 29 and Plate II, figs. 13-19. The other was a polar view but it proved impossible of illustration owing to the overlying of the chromosomes. In each case it could be clearly seen that the two daughter chromosomes were separating to both poles at different points, *i.e.* that each daughter chromosome possessed two points of attachment which were separating to opposite poles. In the side view the identity of the two daughter chromosomes is somewhat difficult to follow in the centre of the cell, but in the polar view the two daughter chromosomes can be clearly traced out along the whole of their length. The two free arms of this chromosome are of different lengths, and it can be seen that each daughter chromosome has one arm of each type, so proving that they (the daughter chromosomes) are derived from a chromosome with two attachments.

There are two ways in which the daughter chromosomes of a chromosome with two points of attachment can separate. Either the two attachments of one daughter chromosome can go to the same pole or they can go to opposite poles (see Text-fig. 30). In the first case each of the daughter nuclei will contain a double attachment chromosome and will be identical with the parent nucleus. In the second case the passing of the two points of attachment to opposite poles puts a large strain on the daughter chromosomes, which become drawn out (see Text-fig. 29 and Plate II, figs. 16-18) and no doubt eventually break, giving nuclei having chromosomes with one point of attachment only, *i.e.* with chromosome complements different from that of the parent nucleus. The chances of either mode of disjunction occurring are equal (see Text-fig. 30) and so the probability of getting a nucleus containing a double attachment chromosome after n cell generations is 2^{-n} . There is therefore a strong tendency for such chromosomes to disappear, and this accounts for the fact that they were not observed in root tips fixed some time after treatment (cf. *Tulipa*). This may, in part at least, account for the non-appearance of such chromosomes in the offspring of *Drosophila melanogaster* heterozygous for the closed X-chromosome (Morgan, 1933).

Two other types of abnormality were seen in *C. biflorus*. In two cases ring chromosomes occurred (Text-fig. 27). It was very difficult to be certain that the ends of the chromosomes were really fused, but since such chromosomes are known to occur, having been indisputably observed elsewhere, it is very probable that the two cases observed in *Crocus* are of this nature. Interlocking of these ring chromosomes, such as Navashin (1930) reports, is not present as the cells containing them were undergoing their first division (4 days after treatment).

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The other type of abnormality, about which there is no doubt, is the occurrence of lateral trabants (Text-figs. 22 and 23 and Plate II, fig. 22). These must have arisen by lateral fusion. Lateral trabants have been seen previously by Darlington (1929) in *Tradescantia* and Levan (1932) in *Allium*. They have also been inferred in *Drosophila* (cf. Stern, 1928), but were too small for cytological demonstration. In one case the lateral



Text-fig. 30. Diagram illustrating the behaviour of chromosomes with two points of attachment. At metaphase separation may begin in two ways, (i) the two attachments on each chromatid may go to the same pole, giving perfect separation of the daughter chromosomes (on the left) or, (ii) the two attachments on each chromatid may go to opposite poles, putting great strain on and leading finally to disruption of the double attachment daughter chromosomes (on the right).

trabant could be seen to be double like the rest of the chromosome, at early metaphase. This has an important bearing on the time of occurrence of abnormalities, and is dealt with more fully later.

Although these lateral translocations always appear as trabants, in several cells where they occurred the normal pair of trabants was also present (Text-fig. 23). Hence the lateral trabants must have originated from an ordinary piece of chromosome and not from a trabant. A trabant is merely a piece of chromosome which is separated by a constriction and which, being too small to assume the normal width of the chromosome,

becomes spherical. Hence these lateral trabants may have originated as small translocations which take on the appearance of trabants. It is very significant that in the three cases where branched chromosomes have been observed the small arm should always be of the order of size of a trabant and separated from the main body of the chromosome by a constriction. In the case of these induced lateral translocations in *C. biflorus* the occurrence of the constriction is doubly significant, since in no case of end-to-end fusion was any sign of a constriction to be seen. Hence the join of a lateral translocation to the chromosome by a constriction seems to be characteristic of the lateral position.

End-to-end fusion is never marked by the formation of a secondary constriction, in *Crocus*, and so the use of constrictions to measure the lengths of translocations, as has been done in the past, is unjustifiable.

Only one tetraploid sector of a root tip was found. This was in *C. Balansae* and must have arisen before irradiation, since a mitosis in it showed the typical distal fragments of the first abnormal division (Text-fig. 15). It appears therefore that irradiation does not produce increased numbers of tetraploid cells.

(ii) *Tulipa Gesneriana*.

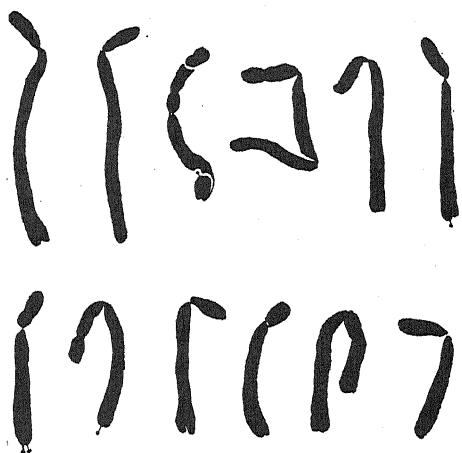
The somatic chromosome number of this species of *Tulipa* is 24. The chromosomes are long and are usually much twisted on the plate, rendering recognition of individuals extremely difficult. It is therefore useless to attempt to obtain an accurate picture of the complement by examination of any one plate. Throughout the examination of one slide any chromosome seen to be flat was drawn separately, and from these drawings it was possible to divide the complement into twelve types, a typical member of each being drawn in Text-fig. 31. It will be seen that the chromosome types are not very distinctive. The trabants are of little use in distinguishing chromosomes, as they are small and only visible in particularly favourable plates. Apparently they are also easily lost during preparation, as in several preparations only one of two chromatids carried a trabant.

Although these chromosomes were drawn from treated material (C 3 was chosen owing to the perfection of the fixation) the frequent occurrence of each type, confirmed during the examination of C 1 in which no obvious abnormality occurs, makes the likelihood of any one drawing representing a translocated chromosome extremely improbable. It is clear from the nature of the chromosome types, however, that small translocations, whether simple or reciprocal, could not be observed with

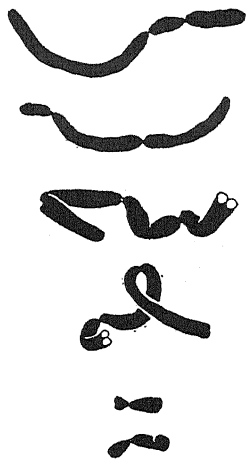
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certainty. A translocation from the long arm of type 1 to the long arm of type 5 could give two chromosomes exactly simulating the two original types.

Examination of a very large number of divisions at full metaphase from all treatments revealed only a small proportion of abnormalities, the most remarkable being the occurrence of two cells containing 23 apparently normal chromosomes and one very long chromosome with two attachment constrictions. The appearance suggests end-to-end fusion



Text-fig. 31.



Text-fig. 32.

Text-fig. 31. Normal chromosomes of *Tulipa Gesneriana* var. Philippe de Commynes. $\times 3000$.

Text-fig. 32. Abnormal chromosomes from irradiated root tips of the same three months after treatment. The top four chromosomes have two points of attachment, the top two being from one cell. The bottom two are proximal fragments. $\times 3000$.

of two chromosomes, which view was strengthened by the attempt (see particularly Plate II, fig. 20) to orientate the original arms of the component chromosomes away from the centre of the plate as in similar chromosomes observed in *Crocus*.

It is probable that translocation involving the major portion of a chromosome, including the attachment, has occurred and that the remaining portion of the translocated chromosome has either been eliminated as a fragment or translocated on to one of the other chromosomes.

In one plate, two double attachment chromosomes occurred together with 20 apparently normal chromosomes (see Plate II, fig. 21).

On two occasions very short chromosomes containing attachments were observed. It was impossible to be certain whether the missing

segments of these chromosomes had been eliminated as fragments or translocated.

Beyond these two chromosomes no clear evidence of fragmentation was seen.

An attempt to find anaphases showing the separation of chromosomes with two attachments was made but was unsuccessful, the high number of chromosomes in *Tulipa* rendering observation difficult.

It is interesting to find chromosomes with two attachments occurring several months after treatment, since their chances of survival are small (see above). The only apparent explanation of their occurrence is that there were a large number of such chromosomes formed as a result of irradiation and that the observed cases represent the surviving ones. Now in *Crocus* in the first few abnormal mitoses after irradiation double attachment chromosomes, although more common than in the *Tulipa* material, were comparatively rare, and indeed in the later fixations no such chromosomes were seen. Hence in *Tulipa* the formation of double attachment chromosomes as a result of irradiation is probably more common than in *Crocus*. It is impossible to say whether fragmentation is necessary before the fusion which gives such chromosomes takes place, but in any case it is a question of end-to-end fusion and this might conceivably be more common in *Tulipa* than in *Crocus* as there are more chromosomes, and consequently more ends, present.

(iii) *General—the types of abnormalities which occur.*

The above observations indicate two things, viz. (i) that the attachment constriction is a constant body and is unaffected by irradiation (this concept is treated more fully in the discussion); and (ii) that the occurrence of abnormalities usually involves two processes, fragmentation and end-to-end fusion, and very occasionally lateral fusion¹. Hence the number of possible abnormalities which can arise as a result of irradiation is limited.

The abnormalities are:

(1) *Proximal fragments*, which are the parts of broken chromosomes containing the point of attachment. They behave in every respect as normal chromosomes and are very common at all times after irradiation.

(2) *Distal fragments*, which are the parts of broken chromosomes not containing the point of attachment. They do not possess the property of

¹ It is impossible to say whether fusion of unbroken ends of chromosomes takes place or not.

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separation at anaphase and consequently, although common in the first cell generations after irradiation, they become increasingly rarer and finally are completely lost.

(3) *Translocations*, which arise as a result of the fusion of distal segments with other whole chromosomes or proximal fragments. They behave like normal chromosomes and are persistent. They are common at all stages.

(4) *Double attachment chromosomes*, which result from the fusion of two whole chromosomes or two proximal fragments or one of each. All chromosomes of this type, actually observed, consisted of two fused proximal fragments. Their anaphase behaviour is characteristic and results in their becoming gradually rarer in succeeding cell generations. They are not very common.

(5) *Ring chromosomes*, which are the product of the fusion of the two ends of a single normal chromosome or proximal fragment. They are very rare, and no indisputable example was observed although two possible cases were present.

In addition to the above types, a few cases of *lateral translocation* of trabants occurred in *C. biflorus*. Such cases were not seen in any other plant and are very uncommon.

The only abnormalities which have a good chance of survival are translocations and proximal fragments. The chromosome complement may also show deletions as a result of the loss of distal fragments.

Working with irradiated dominant pollen used to fertilise normal recessive ovules, Stadler (1931) has shown genetically that only translocations and deletions, as opposed to trisomy, occur. The above observations provide a cytological basis for this. Furthermore, the occurrence of root tips chimerical for different abnormalities demonstrates the impracticability of irradiating somatic structures in order to produce useful single genetical rearrangements.

IV. DISCUSSION.

(i) *The time of division of chromosomes at mitosis.*

There are two schools of thought as to the time of division of somatic chromosomes at mitosis. Robertson (1931) and others have contended that the chromosomes have already split at anaphase for the next division. More recently Hedayetullah (1931) has made observations on the somatic chromosomes of *Narcissus* which he considers demonstrate splitting of the chromosomes at metaphase of one mitosis for the next division. Koshy

(1933) also claims to have demonstrated the same phenomenon in the somatic chromosomes of *Allium*.

In opposition is the view that the chromosomes are unitary at anaphase and telophase and split during the resting stage. This has formed one of the foundations of Darlington's (1931) precocity theory, which regards meiosis as a modification of mitosis, the essential differences being due to the chromosomes splitting during the previous resting stage at mitosis but not until pachytene at meiosis. Darlington (1932) has dealt rather fully with the previous evidence on this point and a repetition would be superfluous. In brief he considers that the observational evidence for a metaphase-anaphase split of the chromosomes is based on an optical illusion, the spiral structure appearing double in optical section, and maintains that the split of the chromosomes at such a stage is mechanically incompatible with observations on the behaviour of the chromosomes at anaphase. The production of abnormal chromosome types by irradiation throws some light on the subject.

There are two possibilities with regard to the relative times of splitting of the chromosomes and the production of abnormal types, viz.

(a) that the production of abnormalities is prior to the time of effective split of the chromosomes;

(b) that the production of abnormalities is subsequent to the effective splitting of the chromosomes.

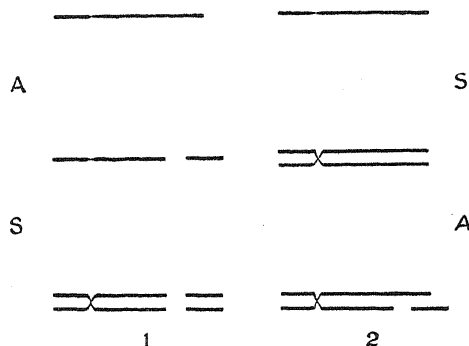
The first relationship would give rise to chromosomes which would be equal in both chromatids (daughter chromosomes) for the abnormality, since the abnormality would be produced as a unit and then rendered double by the subsequent split of the chromosomes. The second type would give rise to unequally abnormal chromatids (daughter chromosomes) in the same chromosome, since owing to the prior split the chromatid and not the chromosome would be the unit for the production of abnormalities (see Text-fig. 33).

Now in *Crocus Olivieri* Stone (1933) has shown that divisions in progress at the time of irradiation are unaffected. A very careful re-examination of the material has confirmed this; all metaphases and anaphases in divisions in progress during irradiation are absolutely normal. Furthermore, while only a few divisions are in progress at irradiation the vast majority of divisions subsequently show abnormalities. Hence the production of abnormal chromosomes must take place in the long enforced resting stage which forms the next phase of the action of X-rays. Therefore if splitting of the chromosomes for any mitosis takes place at metaphase or anaphase of the previous division the splitting of the chromo-

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somes for the first abnormal division would take place during the metaphase or anaphase in progress before or during irradiation and so would be prior to the formation of abnormalities (in many cases prior to irradiation), thus giving unequally abnormal chromatids in the chromosomes at the first abnormal metaphase. No such unequal abnormalities have been observed in any of the three species of *Crocus* examined. Furthermore, Lewitsky and Araratian (1931) observed only one case of unequal chromatids in the whole of their study on X-rayed *Crepis capillaris*. Hence it seems impossible that the chromosomes split at metaphase-anaphase for the next division.

Considering the opposite view, if the chromosomes split during the resting stage before mitosis then they could split after the production of



Text-fig. 33. Diagram illustrating the effects of the two possible relative times of occurrence of abnormality and effective splitting of the chromosomes. *A* = time of occurrence of abnormality. *S* = time of effective split of the chromosomes. (1) The abnormality occurs *before* the chromosome splits and so the abnormal chromosomes always have equal chromatids. (2) The abnormality occurs *after* the chromosome splits and so the abnormal chromosomes have unequal chromatids.

abnormalities and so always give equally abnormal chromatids, as has been observed. The lateral translocation illustrated in Text-fig. 23 is interesting in this respect. The translocation must have occurred before the split of the chromosomes as it is exceedingly improbable that two equal translocations should occur laterally at the same corresponding places on sister chromatids. The singleness of the other lateral translocations is due to their position on the chromosome. Branched chromosomes obviously cannot split properly if the branching occurs in a plane different to that of the split.

The one unequal fragmentation observed by Lewitsky and Araratian was probably due to a slight upset in the time of production of abnormalities or of the splitting of the chromosomes. It is conceivable that this might occur and yet both processes take place during the resting phase.

Thus it is impossible to explain the type of abnormality produced by X-radiation if the chromosomes split at metaphase-anaphase, but the abnormalities entirely fit in with the view that the chromosomes split during the resting stage.

(ii) *The constancy of the point of attachment.*

The properties of the point of attachment, or kinetic body, are very different from those of the rest of the chromosome.

At mitosis the attachment does not divide until the end of metaphase, whereas the rest of the chromosome has split during the previous resting stage. This difference in the time of division determines the whole mechanism of chromosome separation at mitosis. The delayed division of the attachment allows the chromosomes to become orientated in a plane before complete separation into two daughter chromosomes, and hence allows of efficient formation of two daughter nuclei by equal division of the chromosomes.

At meiosis the point of attachment has even more importance. It probably determines the assumption of a spiral structure which, if the torsion theory is correct, is the cause of crossing-over when the threads are weakened by the splitting of the chromosomes at pachytene. It certainly controls terminalisation of chiasmata, which, by rendering chromosome separation at early anaphase easier allows of the simple working of such mechanisms as ring formation, which is of great evolutionary importance in *Oenothera*, *Rhoeo* and other plants. Finally the attachment constriction again allows of the orientation and equal separation of the chromosomes at metaphase-anaphase.

This brief résumé of the properties of the point of attachment, while not pretending to be exhaustive, does emphasise its important functions in determining chromosome behaviour. The body of the chromosome contains the genes, which determine the phenotype of the plant, but the point of attachment is responsible for making the chromosome mechanism a working proposition. Without it a chromosome cannot behave normally.

One would expect therefore that the point of attachment would have an individuality separate from that of the rest of the chromosome. This is borne out by the above observations on induced-abnormal chromosomes. In no case does a free distal fragment without an attachment pass into the daughter nuclei at the first abnormal mitosis. This is equally true of long and short distal fragments. Conversely, in no case does a proximal fragment with an attachment, however long or short it may be, fail to

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separate normally and pass into the daughter nuclei. The real test comes in the formation and behaviour of the chromosomes with two points of attachment. Such chromosomes have no control over the two attachments, which moreover behave independently of one another, and in half the cases separate in a manner that must result in the disruption of the chromosome.

Finally, the number of attachments is never increased or decreased by irradiation. This destroys the notion that the attachment constriction is a point of weakness.

Similar deductions follow from the observations of other workers, particularly Navashin (1931) on irradiated *Crepis tectorum*, Lewitsky and Araratian (1931) on *Secale cereale*, *Vicia sativa* and *Crepis capillaris*, and of Dobzhansky, Painter, Stern, etc. on *Drosophila melanogaster*. In all cases they have shown that translocations, interstitial and terminal, simple and reciprocal, may take place, but that the number of chromosomes, *i.e.* of attachments, is not altered.

Opposed to this are the observations of Darlington (1929), Navashin (1926) and Sveshnikova (1929). Darlington claims to have shown the *de novo* origin of fragments at meiosis in *Tradescantia*, and their persistence until at least the first microsporocyte division. Navashin found a plant of *Crepis tectorum* which showed breakage of one chromosome with persistence of the two fragments, and Sveshnikova studied two races of *Vicia Cracca* which apparently differed by a simple fragmentation. In none of these cases is it proved, except perhaps in *Tradescantia*, that the fragments arose by simple breakage with subsequent formation of an attachment; therefore the evidence is not conclusive.

Fusion can occur as a simple process if the two points of attachment fuse, and this may be the origin of the attached X-chromosomes in *Drosophila melanogaster*¹ and may be connected with the origin of some of the Acrididae (Darlington, 1932) and of certain Reptilia (Matthey, 1931). In both cases the attachments are terminal or nearly so. Consequently these examples fall into line with the concept of the constancy of the number of points of attachment.

It may be taken as proved therefore that the point of attachment is a constant body with properties and an individuality distinct from those of the rest of the chromosome.

¹ In this case the small but regularly occurring proportion of detachments of the two X-chromosomes is explicable as being due to the points of attachment not behaving entirely as one but occasionally going to opposite poles.

(iii) *Fragmentation and fusion as evolutionary processes.*

The importance of polyploidy as an evolutionary process is now generally accepted, but in most natural orders and indeed in some genera there exist polyploid series on more than one basic number. Obviously an explanation for this other than polyploidy must be sought.

There are two possibilities, viz. that the different basic number arose by fragmentation and fusion, or that it arose by the attainment of a secondary balance.

With the latter process we are not here concerned. It is sufficient to note that secondarily balanced forms have been produced experimentally in the genera *Nicotiana* (Lammerts, 1932) and *Crepis* (Collins and Mann, 1923) and that there is evidence of the secondarily balanced nature of the Pomoideae (Moffett, 1931) and of *Dahlia Merckii* (Lawrence, 1931 a).

Fragmentation and fusion have been considered to be important factors in chromosome evolution, particularly by animal cytologists. The numbers and morphology of the chromosomes of *Crocus* seem to be explicable only on the basis of fragmentation and fusion (Mather, 1932), and other plants such as *Cardamine pratensis* (Lawrence, 1931 b) and *Nicotiana* spp. (Vilmorin and Simonet, 1928) possess chromosomes which have apparently resulted from fusion. These processes have in the past been considered simple, but Navashin (1932) has emphasised that, in view of the constancy of the number of points of attachment in a chromosome complement, they cannot be as simple as has been thought. The present observations lend considerable support to Navashin's arguments, and consequently fragmentation and fusion can only be considered to occur in a complicated manner. Navashin has put forward a hypothesis to explain their occurrence, but it involves aneuploidy of one chromosome (to introduce an extra point of attachment) followed by a number of translocations. Such a process must take several generations to occur and it is therefore unlikely that it would go on in a sexually reproducing plant, owing to the tendency of the sexual process to eliminate chromosome abnormalities (cf. Darlington (1929), who attributes the rare occurrence of fragments in the great Dicotyledonous genera to that cause). In an asexually reproducing organism it is conceivable that fragmentation and fusion, along the lines suggested by Navashin, have occurred, and indeed in *Crocus* it is very probable that these have gone on.

Fusion, as pointed out above, may occur as a simple process where two chromosomes with terminal or nearly terminal attachments fuse at the attachment end, as has probably occurred in *Drosophila melanogaster* to

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give the \widehat{XX} line; but the comparative rarity in plants of chromosomes with terminal (or nearly so) attachments renders this possibility of little importance. Finally it is conceivable that very occasionally an attachment may be formed *de novo* or may be lost, but these cases must be exceedingly rare if indeed they occur at all.

Fragmentation and fusion, we may therefore conclude, are to be considered as of evolutionary importance in a very few cases only, and since the attainment of a secondary balance as a means of originating new basic numbers has occurred experimentally and has further been inferred in other groups of plants, any apparent case of fragmentation or fusion must be very carefully scrutinised before its validity is admitted.

V. SUMMARY.

1. The first types of chromosome abnormality to arise as a result of irradiation by X-rays are fragmentation and end-to-end fusion.

2. Chromosome fragments, however long or short, if not carrying the point of attachment of the original chromosome do not orientate themselves upon the equatorial plate at the metaphase of the first abnormal mitotic division, nor do they separate to the poles at anaphase of this division. These fragments do persist if they fuse with a chromosome or a fragment carrying an attachment.

3. Chromosome fragments, however long or short, which do carry the point of attachment, behave entirely normally at metaphase and anaphase.

4. Chromosomes with two points of attachment at both metaphase and anaphase are described. In half the cases the anaphase separation of these chromosomes is such that they must break.

5. The number of points of attachment in the chromosome complement never varied as a result of irradiation in any of the plants studied.

6. Lateral translocations, in the form of lateral trabants, occur in *C. biflorus*. Attention is called to the fact that wherever branched chromosomes have been seen they always appeared as chromosomes with a lateral trabant.

7. After four or five days the chromosome complement shows only translocations and (probably) deletions as a result of irradiation; this is due to the instability of the other types of abnormality. The abnormalities which persist give rise to chimerical sectors in the roots.

8. Evidence is adduced from the observations to show that the chromosomes split effectively during the resting stage for the next mitosis.

9. The concept of the constancy of the number of points of attachment in a chromosome complement, and its bearing on the evolution of chromosome complements, are discussed.

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EXPLANATION OF PLATES I AND II.

(Photographs taken by Mr H. C. Osterstock.)

PLATE I.

- Figs. 1-6. Photos at gradually descending foci of anaphase in a root tip of *C. Balansae* five days after irradiation. Note the behaviour of the distal fragments in Figs. 2-4, which neither orientate themselves nor separate (cf. Text-fig. 16). \times circa 2500.
- Figs. 7-12. Photos at gradually descending foci of a metaphase in a root tip of *C. Balansae*, three days after irradiation. In Figs. 9 and 10 note the chromosome with two attachments and also how only *four* other chromosomes are orientated on the metaphase plate. In Fig. 12 note the distal fragments off the plate (cf. Text-fig. 25). \times circa 2000.

PLATE II.

- Figs. 13-19. Photos at gradually descending foci of a somatic anaphase in a root tip of *C. biflorus* four days after irradiation. Note the behaviour of the double attachment chromosome, and of the distal fragments (cf. Text-fig. 29). \times circa 2000.
- Fig. 20. Somatic metaphase from irradiated *Tulipa Gesneriana* var. Philippe de Commines, showing a chromosome with two points of attachment (arrows mark the two attachments). Note the orientation of this chromosome. \times circa 1300.
- Fig. 21. Somatic metaphase from irradiated *Tulipa Gesneriana* var. Philippe de Commines showing two chromosomes with two points of attachment (arrows mark the two chromosomes). There are only 20 other chromosomes. \times circa 1500.
- Fig. 22. Somatic metaphase of irradiated *C. biflorus* showing a lateral trabant (marked by arrow). \times circa 2000.



Fig. 1.



Fig. 2.

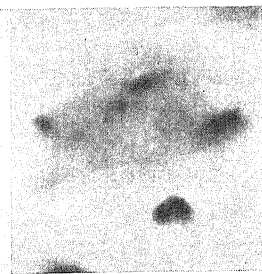


Fig. 3.

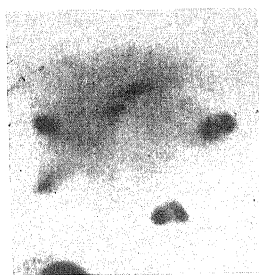


Fig. 4.



Fig. 5.



Fig. 6.

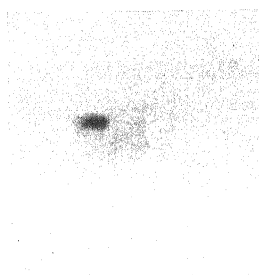


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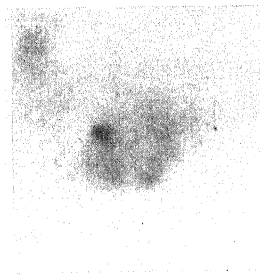


Fig. 8.

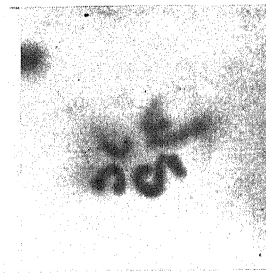


Fig. 9.

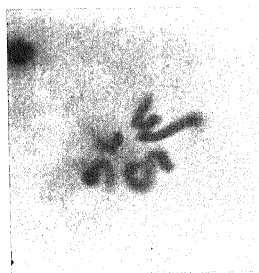


Fig. 10.

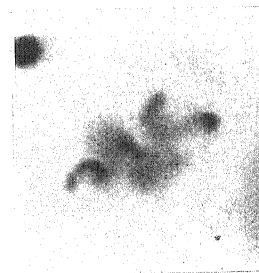


Fig. 11.

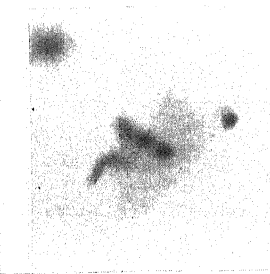


Fig. 12.

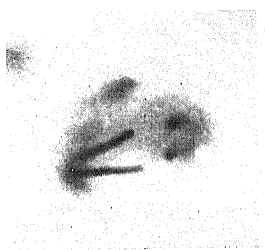


Fig. 13.

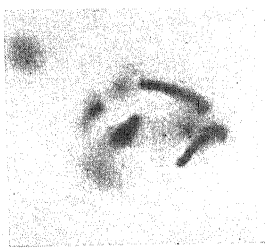


Fig. 14.



Fig. 15.



Fig. 16.

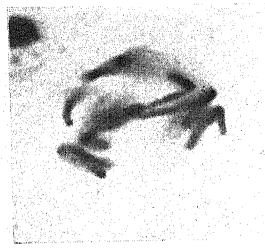


Fig. 17.

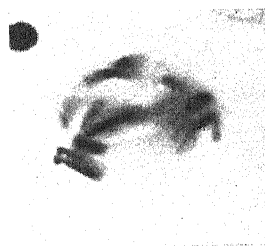


Fig. 18.

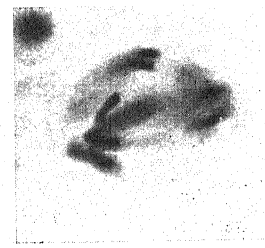


Fig. 19.

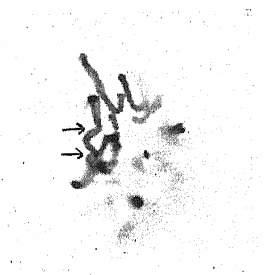


Fig. 20.

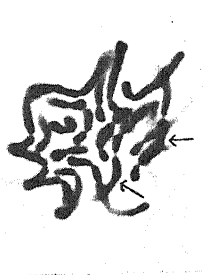


Fig. 21.

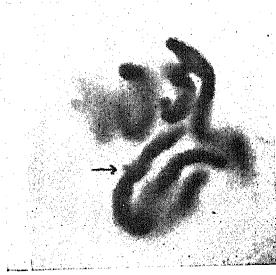


Fig. 22.

A LETHAL IN THE RAT.

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AND S. K. KON

(National Institute for Research in Dairying, Reading).

(With Three Text-figures.)

IN the National Institute for Research in Dairying a colony of rats is maintained. The rats are the "Japanese" of the fancier, the "hooded" of Castle. A year or so ago it was noted that in the case of a number of litters some of the pups failed to continue to develop normally. As time passed it became clear that some unsuspected factor was operating with great regularity. Perfectly normal litters would be born and remain normal until the second week of lactation, when it would be found that one or more of the pups was losing weight. The amount of milk visible in the stomachs of these pups rapidly diminished, and after a few days this organ looked quite empty. Their weight decreased progressively and rapidly until finally they died, apparently of starvation and in a condition of marked emaciation. These abnormal pups appeared most frequently amongst the progeny of one particular male that had been used extensively for breeding. In view of this fact, and because it was quite certain that faulty nutrition or husbandry generally could not be held responsible for the death of these pups, an analysis of the very complete and detailed records of the rat colony was made.

There were 65 litters which included 649 offspring, and of these 567 (280 ♂♂: 287 ♀♀) were normal, and 82 (43 ♂♂: 39 ♀♀) were abnormal. By abnormal is meant that the individual began to lose weight in the second week of lactation and thereafter, within a few days, died, apparently of inanition. These matings had not, of course, been planned as part of a genetical experiment, and involved a number of individuals which, as far as the records went, had never been associated with the production of abnormal pups. Further, it is the custom in the N.I.R.D. to reduce all litters to six immediately after birth, and since the abnor-

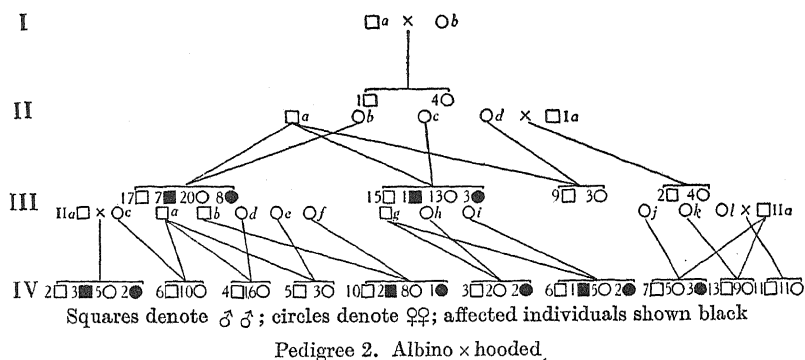
mality is not expressed until the end of the first week, amongst those who were rejected would be both normals and abnormals. This procedure, however, would not be expected to affect in any significant fashion the relative distribution of normality and abnormality. The fact that among 390 pups (65 litters each of 6) there were 82 abnormals permitted us to assume that the abnormality, probably, was of the nature of a genetic recessive character, and that we were dealing with the first lethal to be recognised in the rat.

It is indeed of interest to note that no lethal in the rat has so far been recorded. This does not necessarily mean that in this species lethals are not as plentiful as they are in the mouse. In a general way it is safe to assume that the number of varieties of a domesticated species is an indication of the frequency of mutation in that species. It is equally reasonable to assume that if mutation generally is fairly common, then mutations of a lethal kind are also common. There are more "varieties" of the mouse than of the rat. But this is probably nothing more than a reflection of the fact that there are many more "fanciers" of the mouse than of the rat, and by the fancier mutations are quickly observed, and commonly welcomed, for he seeks ever to perpetuate novelty. Physiologists, on the other hand, more commonly have used the rat as experimental material, and in this the physiologist desires uniformity and stability. So it is that in the past the geneticist has received more problems and more material from the fancier than from the physiologist. A lethal, if and when it became expressed, would puzzle the fancier and he would seek help, but it would merely annoy the physiologist and he would kill off his stock. But apparently a welcome change is taking place, for to-day physiologists do not forget that a phenomenon which seems to vitiate their own experiments may easily prove to be the means whereby a kindred science may be advanced.

If it is assumed that this abnormality is indeed an autosomal monogenic recessive character, then it would be expected that abnormals would appear in the following proportionality:

1 abnormal in a litter of 6	0.4330	times
2 abnormals	0.3609	"
3 "	0.1604	"
4 "	0.0401	"
5 "	0.0053	"
6 "	0.0003	"
	1.0000	

The following table gives the distribution of abnormals as they were observed and as they would have been expected:



Amongst the litters which included abnormals and which correspond to the F_2 generation there appeared 19 abnormals (8 ♂♂: 11 ♀♀) among 84 individuals (40 ♂♂: 44 ♀♀), a close approximation to the expected 21, the condition being regarded as an autosomal monogenic recessive. Amongst all the litters which included abnormals and which, therefore, presumably were produced by the mating of two heterozygotes, 35 abnormals (14 ♂♂: 21 ♀♀) appeared amongst 151 individuals (74 ♂♂: 77 ♀♀), again a close approximation to the expected 37.7. It is concluded, therefore, that this lethal is indeed an autosomal monogenic recessive. Manifestly it could not be an autosomal dominant and the pedigrees show quite conclusively that it is not a sex-linked recessive. Male III *b* and female III *f* were extracted albinos, and now we have a line of these in which the lethal is present.

DESCRIPTION OF THE ABNORMALITY.

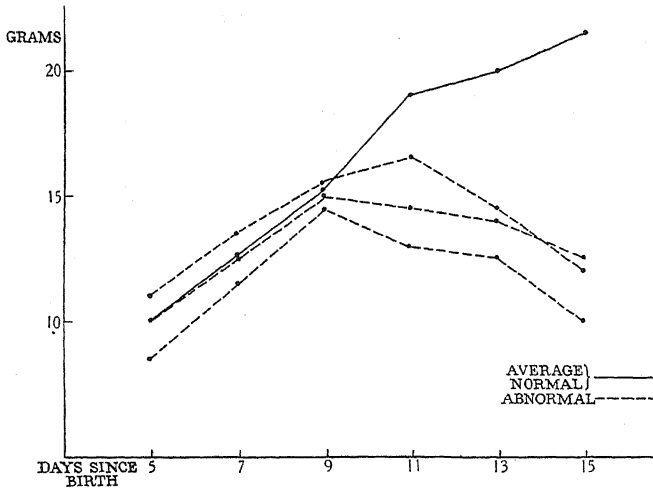
Post-mortem examination has failed to reveal any gross abnormality of structure. The abnormal pup is not to be distinguished from the normal before the second week of lactation, and the first indication of the condition is that the weight becomes stationary and that loss of weight thereafter occurs. Amongst 50 abnormal pups the first discernible loss of weight was

In	9 cases on the	9th day
15	"	10th "
20	"	11th "
6	"	12th "

The average weight of the individual pups in the litter at the time immediately before the loss of weight on the part of the abnormals is first discernible is 14 gm. Thereafter, whilst the normals increase, their abnormal litter-mates decrease so that at the time when the normals

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average 18 gm. the abnormal average only 12, and the weight of the abnormal at death is about 10 gm. The graph given below illustrates the relative growth rates of the average normal and of the three abnormal in a certain litter.



Graph I

In an attempt to show that the fault lay with the pup and not with the mother, the litters which were expected to include abnormal and also others which were known to include abnormal were reduced, part of the litter being given to a foster mother of known quality. But although with the smaller litter the growth-rate of the individual was more rapid, always between the 9th and the 12th days the weight of the abnormal became stationary. Thus it appears that it is the time or the stage in the development of the individual which is the important factor, and not the rate of growth during the first week. The average duration of life after the first recognition of the abnormality was 5-17 days in the case of 50 rats. When the abnormality is first recognised it is to be noticed that the abnormal pups are much more restless than are their litter-mates. They appear very hungry and drink eagerly from a pipette. But, though milk and warmth be given, they become weaker and are unable to sustain the effort of suckling and more frequent intervals between feeding become necessary. Until they become moribund the sucking reflex remains. With increasing loss of weight the head comes to look disproportionately large and the bodies appear to be dehydrated. Defaecation and urination occur, but the amount of urine passed is small. The faeces appear normal. There is an absence of body fat, and for this reason, probably, the "hiber-

nating gland" in the scapular region stands out clearly and appears to be hyperaemic. Films of blood and bone marrow show no definite signs of a primary anaemia. The marrow is active but not abnormally so, and the blood picture differs from the normal, as far as we can judge, only in the absence of a physiological leucocytosis. Some polychromasia is noticeable in the red cells. There is no eosinophilia. Death would appear to be due to inanition, but the primary cause of this so far escapes us.

Though the week-old rat is not a convenient material for the detailed study of faulty function, it should not be impossible to determine the primary cause of death, for now that the genetics of the condition has been demonstrated and the genotype of many animals determined, it is always possible to provide a sufficient number of abnormals for systematic examination.

For the reason that this lethal in its action leads to the death of the individual shortly after birth it is to be regarded, according to the current classification, as a sub-lethal. Now, whilst there can be no disagreement with the division of lethals into gametic and zygotic, into dominants and recessives, the attempted distinction between an absolute lethal, *i.e.* a zygotic lethal which operates antenatally, and a sub-lethal which leads to death at the time of, or shortly after, birth cannot command the same general approval. Such a classification seems to imply grades of lethality, differences on the part of different genetic factors in respect of the power to kill. Furthermore, it gives to birth an importance which this event cannot claim. A lethal factor is one which in its action produces abnormality of a kind or degree as to be incompatible with continued life. It is the character that is lethal and not the gene itself, and since different characters, or the same characters in different species or varieties, are expressed at different times during ontogeny, it follows that death will not always and in all forms occur at the same time with reference to some such salient point as conception or parturition.

If, for the purposes of argument, it is assumed that the developmental histories of a kangaroo and of a guinea-pig are identical, and that in both the same lethal operates, then, because so much of the development of the individual occurs before birth in the guinea-pig and after birth in the marsupial, one and the same gene could be a sub-lethal in one and an absolute lethal in the other although it affected the same mechanism and at the same point during ontogeny. Furthermore, what is the relation of hatching to birth, and are all zygotic lethals in the bird absolute or sub-lethals? Manifestly, this attempted classification is not helpful and therefore should be abandoned.

For the classification of lethals the suggested scheme of Haldane¹ would appear to possess great advantages, but until the embryologist has given thought to the matter, to construct such a classification is impossible. For the present it must suffice to distinguish between those lethals which so distort development that normality is never expressed, and those which, after development has been completed, transform pre-existing normality into abnormality.

Disregarding such agencies of mortality as deficiency and abnormalities in the distribution of the chromosomes, it may be accepted that a lethal is a genetic factor which (1) prevents development of the gamete or zygote for the reason that it represents the absence of some ingredient essential to normal development, or on the other hand for the reason that it introduces in the developmental processes some ingredient not to be found in normality; or (2) so modifies a previously existing and normally functioning character in the zygote as to render it grossly abnormal. Thus lethals can be classified according to the time at which their effects become evident; there are those which operate during the phase of development, during gametogenesis or, in the zygote, between fertilisation and maturity, and there are those which produce their effects after maturity (of the individual as a whole or of a part thereof) is passed, to cause a breakdown in some organ or organ-system essential to life and so to lead to disharmony and death.

Surely any gene that in its action leads to the death of the individual is a lethal: it matters not when it kills, whether *in utero* or in old age. Are not haemophilia which kills off the female *in utero*, juvenile amaurotic idiocy, glioma of the retina or myositis ossificans progressiva which destroy the adolescent, Huntingdon's chorea and arteriosclerosis which kill the fully grown, examples culled from human pathology, equally lethal? Doubtless it is because, according to our code, it is more reprehensible to kill an infant than a dotard that the term lethal thus far has been reserved for those factors which destroy *in utero* or about the time of birth, but we err when we ascribe moral qualities to the gene.

SUMMARY.

The existence of a monogenic autosomal recessive lethal in the rat is demonstrated. Death occurs, apparently from inanition, during the second week of lactation.

¹ Haldane, J. B. S. (1932). "The time of action of genes and its bearing on some evolutionary problems," *Amer. Nat.* **66**, 5-23.

A STUDY OF DOMINANT MOSAIC EYE-COLOUR MUTANTS IN *DROSOPHILA MELANOGASTER*.

II. TESTS INVOLVING CROSSING-OVER AND NON-DISJUNCTION¹.

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(With Plates V—VI and Six Text-figures.)

In the preceding paper of this series (Glass, 1933), the phenotypes of the six allelomorphs of brown which have been studied, the two Moiré allelomorphs, and Grape, the allelomorph of pink, all of which are dominant mosaic eye colours, produced by X-rays in *Drosophila melanogaster*, were described. To supplement these brief descriptions, in themselves quite inadequate to give the reader a true conception of the appearance and nature of these unique forms, the accompanying plates have been prepared with the kind and able assistance of Miss Mörrck, the artist at the Anatomical Institute of the University at Oslo, Norway. Particular care has been taken to select typical and representative individuals, and to give as perfect a reproduction as possible. The reader is referred to the legends accompanying the plates for more explicit descriptions of the several types reproduced.

In the present paper is presented in addition the detailed evidence bearing on the loci of the genes in question, the associated chromosomal abnormalities, the effects of the latter upon crossing-over and, in the case of the mutual translocations, a study of the non-disjunction occurring. (It has already been pointed out that in every case there is present, and associated with the eye colour, a chromosomal abnormality, either an inversion or a mutual translocation, or some combination of both.)

The same symbols will be used in this paper as in the preceding one, i.e. for the allelomorphs of brown the abbreviated forms *V1*, *V2*, *V3*, etc. For Grape the symbol *Gr* will be used as an abbreviation of *p^{Gr}*. The symbols *Mo1* and *Mo2* stand for the Moiré allelomorphs.

¹ This paper was constructed from the original thesis in genetics, presented to the faculty of the Graduate School of the University of Texas in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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TESTS INVOLVING PLUM (VI).

Plum was crossed to *S/Cy D/C_{IIIx}*¹, and the *F*₁ *VI/Cy D* males were crossed to normal females. Plum showed random assortment with *Dichaete*, and 100 per cent. segregation from *Curly*, facts which show that it is located in the second chromosome, and that there is no translocation between II and III present. Plum showed no sex-linkage; so that there is no translocation between II and the X-chromosome.

Crossing-over in the second chromosome.

VI/Cy females were crossed to *Brisple/Cy* males. "Brisple" is a multiple stock containing the characters *aristaless* (*al*—0·0), *dumpy* (*T^{dp}*—13·0)², *black* (*b*—48·5), *Bristle* (*Bl*—54·7), *curved* (*c*—75·5), *plexus* (*px*—100·5), and *speck* (*sp*—107·0), all in the second chromosome. This stock was especially prepared for the purpose of studying crossing-over in flies having the mutant dominant mosaic eye colours. Unfortunately, in the stock as first prepared, black was not present; this makes the data in column 1, Table I, somewhat different from those in the other two columns, and is the explanation for the parentheses placed around the symbol for black wherever it occurs. The results of the classification of the offspring derived from back-crossing *VI/Brisple* females to *apl/Cy* males (*apl* contains *al dp b pr c px sp*; prepared by Bridges)³ are given in Table I. The data in columns 1 and 2 are from the same type of back-cross, but the cultures in column 1 are mass cultures, while column 2 consists of thirty-seven pair cultures. (Pair cultures giving less than fifty offspring were eliminated.) In the data given in column 3 black was present, as in column 2, but not in column 1; while in addition there was present a virtually non-cross-over *Dichaete DC_x* (the "*C_x*" having been produced by Oliver after X-raying *Dichaete* flies) in the third chromosome coming from the *Brisple/Cy* parent. Both *D* and *Cy* flies were discarded in the count.

¹ These genes are *Star* (*S*—1·3—II), *Curly* (*Cy*—in the left arm of II, and associated with two inversions *C_{III}L* and *C_{III}R*, which prevent crossing-over in II), *Dichaete* (*D*—40·4—III), and *C_{III}x*, which contains an inversion in each arm of III, prepared by Muller using X-rays, for the purpose of preventing crossing-over.

² *T^{dp}* represents *dumpy*, a recessive allelomorph of the dominant *Truncate* (*T*), which has the priority in the choice of a symbol for the locus. Genetic nomenclature is not at present well adapted for such cases, giving a misleading impression regarding dominance in the case of *dumpy* (brown and its dominant allelomorphs illustrate the converse difficulty). Hence, in the present paper, we are using the symbol *dp* to represent *dumpy*.

³ *pr*—the recessive eye colour purple (54·5—II).

A study of Table I shows that only three apparent single cross-over individuals were found, one which was *al V1* and two which were *al dp V1*. Since the *apl/Cy* stock used in collecting the data in columns 1 and 3

TABLE I.

$$P_1: V1/Cy \text{ } \text{\textcircled{f}}\text{\textcircled{f}} \times al \ dp(b)Bl \ c \ px \ sp/Cy \text{ } \text{\textcircled{m}}\text{\textcircled{m}};$$

F_1 back-cross: $al\ dp(b)Bl\ c\ px\ sp/V1\ \text{♀}(\text{♀}) \times al\ dp(b)pr\ c\ px\ sp/Cy\ \text{♂}(\text{♂})$.

Or, for column 3:

$P_1: al\ dp\ b\ Bl\ c\ px\ sp/Cy\ DC_x/+ \ \sigma\sigma;$

$$F_1 \text{ back-cross: } \frac{al\ dp\ b\ Bl\ c\ px\ sp}{V1} \text{ }_{\text{♀♀}}^{+} \times \frac{al\ dp\ b\ pr\ c\ px\ sp}{Cy} \text{ }_{\text{♂♂}}$$

	<i>al</i>	<i>dp</i>	<i>b</i>	<i>Bl</i>	<i>c</i>	<i>px</i>	<i>sp</i>
	1	2	3	4	5	6	7
	VI						
	(Cy and D flies discarded.)						
						1	2
0	<i>al dp(b)Bl c px sp</i>					429	480
	<i>VI</i>					765	726
1	<i>al VI</i>					1	—
2	<i>al dp VI</i>					—	1
1.2	<i>al(b)Bl c px sp</i>					14	9
	<i>dp VI</i>					25	21
1.3	<i>al Bl c px sp</i>				(Incl. in 1.2)	4	15
						12	23
1.4	<i>dp(b)Bl VI</i>					—	2
2.3	<i>al dp Bl c px sp</i>				(Incl. in 0)	11	26
	<i>(b)VI</i>					24	56
2.4	<i>al dp c px sp</i>					1	1
	<i>(b)Bl VI</i>					—	3
2.5	<i>(b)Bl c VI</i>					—	1
3.4	<i>Bl VI</i>					—	1
4.5	<i>al dp(b)Bl px sp</i>					3	4
	<i>c VI</i>					—	7
4.6	<i>c px VI</i>					1	1
1.2.4.5	<i>al(b)Bl px sp</i>					—	1
1.3.4.5	<i>al Bl px sp</i>					—	1
2.3.4.5	<i>al dp Bl px sp</i>					—	1
	<i>(b)c VI</i>					—	4
2.3.5.6	<i>al dp Bl c sp</i>					—	1
	+					—	1
	Total					1239	1303
							1091

contained also Deformed eye (*Df*—47·5—III), which was disregarded, but which was noticed to reduce the length of the aristae, it is probable that the flies were not really aristaless, but respectively *V1* and *dp V1*. The individual recorded in column 2 as *al dp V1* became stuck in the food of the vial in which he was placed for testing, and died without

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leaving any offspring. All the other cross-over individuals found represent the occurrence of double crossing-over, in either right or left arms of II, or in both simultaneously. The regions involved are not relatively long, since no region exchanged was found to include more than three of the markers, and of those of that length there were only three individuals, namely, two which were *dp b Bl VI* and one which was *b Bl c VI*. As is shown below, this third individual was probably really a quadruple cross-over, and not a long double. Had Plum contained only a single long inversion, undoubtedly the type of double-cross-overs involving longer sections would have been most frequent. But these are absent, and hence we must suppose that Plum actually contains two inversions, one in each arm. The data reveal the fact that *Bl* is nearly always found, in double-cross-over individuals, with *b*, and only once with *c*. It follows that the left inversion involves the region including the loci *dp*, *b*, and *Bl*; while the right inversion involves that containing the loci *c* and *px*. The single *b Bl c VI* individual must, in such a case, be the result of a quadruple cross-over rather than of a double.

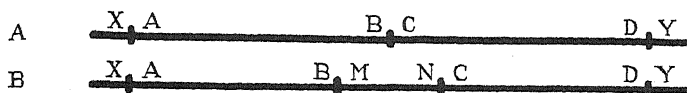
Recent evidence obtained on the interaction of Plum with light (*lt*—55.0—II), by Schultz (unpublished), and confirmed by the writer, in which it is apparent that *lt* affects Plum in the heterozygous state, as though it were an allelomorph of Plum, indicates that one of the breaks in the second chromosome of Plum is at the locus of *lt*. A preliminary cytological survey of Plum by Painter (unpublished) showed that one of the autosomes has a sub-terminal spindle-fibre attachment, instead of the normal median attachment. Dobzhansky (by letter) informs me that he has cytological preparations of Plum in which one of the autosomes appears as a long straight rod. Since, according to the evidence presented by Rhoades (1931), light, contrary to its present position on the map of II, is actually to the left of the spindle-fibre attachment; and since, from our reasoning above, we have seen that *Bl*, only one genetic unit to the left of *lt*, is included in the left inversion, this cannot contain the spindle fibre, which must accordingly be included in the right-hand inversion. The data can then be accounted for on the supposition of three breaks in II, one between *al* and *dp*, one at the locus of *lt*, and the other at the locus of *bw* (104.5). The last is deduced from the fact that Plum shows allelomorphism with *bw*, that it (*VI*) never undergoes recombination with *sp*, and that in the cases where dominant allelomorphs of *bw* are associated with mutual translocations, a break is always present between *px* and *sp*.

A comparison of the several sets of data included in Table I leads to

another striking result. Although the data given in columns 1 and 2 were obtained under different environmental conditions (column 1 consisting of mass cultures and column 2 of pair cultures; raised at different times without special effort to obtain uniformity of conditions), yet they show a striking agreement in crossing-over relationships. But when we examine the data in column 3 (mass cultures), we find a far greater amount of crossing-over occurring throughout the inverted sections, together with a number of quadruple cross-over individuals not found in the other two sets of data. This difference is associated with the presence of an inversion (the above-mentioned DC_x) in the third chromosome, an autosome which is quite unconnected (by translocation) with the second. Further tests are being made to determine whether this phenomenon is of general occurrence, and whether there is actually better synapsis between an inverted chromosome and a normal homologue when synapsis between other pairs of chromosomes is simultaneously hindered. This is of course quite a different thing from the increase of crossing-over observed in the case of *mutual translocations* when, crossing-over being observed in one chromosome, it is simultaneously hindered in the other (Dobzhansky (1931*a*); Dobzhansky and Sturtevant (1931); see also present paper, sections on mutual translocations (*V3, V4, V5, Gr*) and General Discussion).

Theoretical considerations on the origin and synaptic behaviour of inversions, based on Plum.

When there are two separate inversions, there may be either three breaks, as in the case of Plum, making two regions to be considered with respect to the end regions, which we may regard as fixed (Text-fig. 1A); or there may be four breaks, making three regions to be so considered (Text-fig. 1B). It can be shown mathematically that there are eight



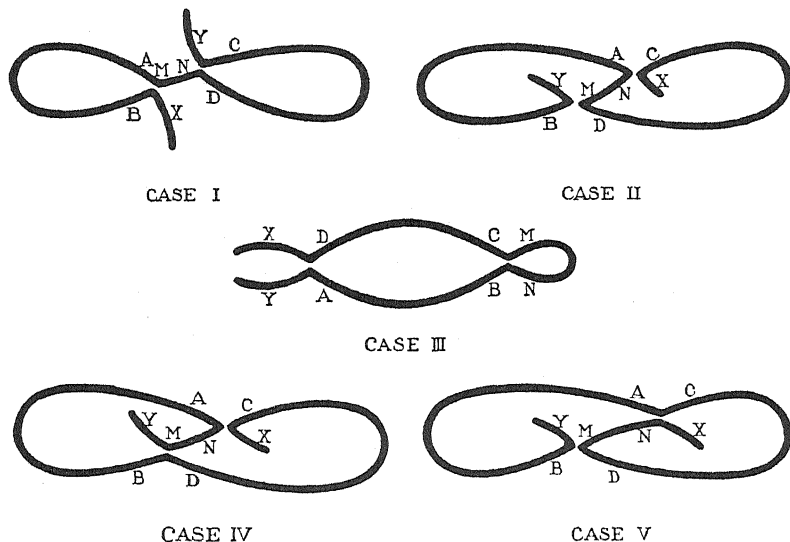
Text-fig. 1.

possible ways in which two regions, each capable of inversion, may be linearly arranged with respect to the fixed end regions; and forty-eight possible ways for three such regions to be arranged. Of these, three of the former class, and four of the latter, may be discarded as being either the original arrangement or involving rearrangements simpler than those under consideration here. There remain five rearrangements for two

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segments with respect to the fixed end regions, and forty-four for three segments.

But if we try to apply the idea of Serebrowsky (1929), that the breaks occur at places where the looping chromosomes touch, the three-break group of cases becomes virtually impossible¹, and for the remainder we obtain only five possible arrangements, as may be seen from Text-fig. 2. Since the only manner in which Plum, from the above data, could be included in the four-break group would be for two breaks to have occurred between *Bl* and the spindle-fibre attachment, a happening most highly



Text-fig. 2.

improbable, in view of the shortness of that distance (0.4 of a genetic unit), we may regard the case as strongly indicative of the untenability of the hypothesis. But Serebrowsky's hypothesis is based on an analogy between crossing-over and the formation of inversions or translocations; in fact, we might regard such chromosomal abnormalities as being the result of non-homologous crossing-over, of which McClintock has brought convincing cytological proof in *Zea* (1932). And from Table I we see that there are ten cases in which crossing-over has occurred between *Bl* and the end of the inversion in which it is located, *i.e.* presumably at the locus of *li*. Furthermore, we know that cytologically these regions immediately to the right and left of the spindle fibre are much longer than their

¹ Because the *three* loci at which the breaks occur must coincide, *i.e.* the chromosome must bend in such a way that it crosses itself twice at precisely the same spot.

genetic length, based on crossing-over, indicates. The case is therefore not to be considered conclusive, at least for the present.

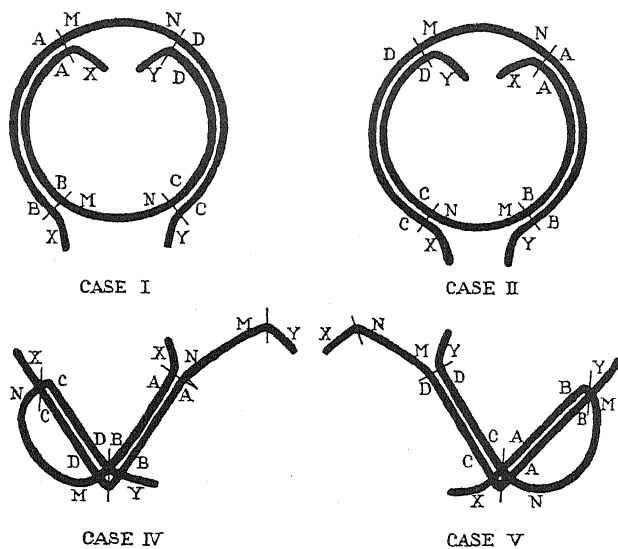
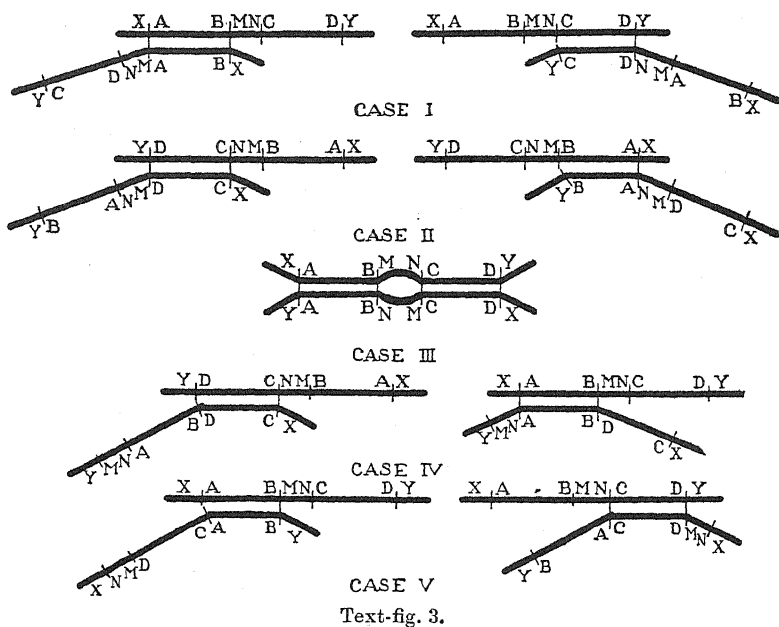
Referring again to Text-fig. 2, we see that if the spindle-fibre attachment lies in the region MN , cases IV and V produce a chromosome with a sub-terminal attachment. But hypothetically, the location of the spindle fibre in the above cases may be in any one of the five regions; and should it be near B or C in the regions AB or CD respectively, cases I and II would also give sub-terminal spindle-fibre attachments. If it can be shown that all cases of double inversions occurring fall into these five types, and that the other thirty-nine (44-5) never occur, we have presumptive proof for Serebrowsky's hypothesis. One clear case to the contrary will make it untenable. Perhaps among the inversions reported by Van Atta (1932 *a*, 1932 *b*), in which sub-terminal spindle-fibre attachments also occur, or among other known cases of double inversions, one may be found in which the homozygote is not lethal, and in which the chromosome can therefore be mapped with greater ease than in the case of Plum¹.

The manner of synopsis of such inverted chromosomes is best shown by means of a diagram. In Text-fig. 3 are illustrated the possibilities in the five cases of Serebrowsky's hypothesis; space will not permit the illustration of the remainder, but they are very similar, and may be constructed in the same way (see also Sturtevant, 1926, 1931). Cases I, II and IV will fit the data found for Plum. Case III will not fit the data, since double cross-overs of the longer type, as described above for possible single inversions, would be expected from it in greater numbers than those involving only short distances. Case V will not fit, since it does not give a sub-terminal spindle-fibre attachment, when the latter, as in Plum, is included in the CD inversion near point C .

The presence of quadruple cross-over individuals in the data presented in Table I, column 3, necessitates the synopsis of inverted and normal chromosomes in the form of a ring, or of a ring with a straight portion appended, as shown in Text-fig. 4 for the cases possible in the Serebrowsky hypothesis applied to Plum. Such types of synopsis would be just as necessary in any of the other thirty-nine cases. This recalls the interpretation placed by Sturtevant on some cross-over individuals observed by Muller in his inversion C_{III} (Sturtevant, 1926; Muller, 1918).

The percentage of double cross-overs in the left arm is, for column 3,

¹ The Serebrowsky theory here discussed may be considered to be a form or derivative of the original idea of non-homologous segmental interchange proposed by Belling (1924 *et al.*).



Text-fig. 4.

16.0 per cent.; in the right, 5.0 per cent. It seems to me justifiable to calculate these percentages on the total of column 3, rather than that of all tests, since the sets of data do not seem, as pointed out above, to be comparable. The proportion of quadruple cross-overs to be expected, if there is no interference whatever, is therefore 16 per cent. times 5 per cent., or 0.8 per cent. The actual percentage found is however also 0.8 per cent., or to be exact, 0.82 per cent., a value which shows there is little or no interference in the case of ring synapsis.

Bridges and Morgan (1923) have suggested that the distribution of crossing-over with respect to the ends of the chromosomes might be symmetrical if synapsis begins at the ends of the loops (seen in pachytene) and progresses towards the mid-point; or if it begins at the tip of the loop and progresses towards the ends. Much discussion has been undertaken as to which of these two possibilities is the right one. Graubard (1932), working with inversions, comes to the conclusion that it must begin near the spindle-fibre and progress outwards. Kikkawa (1932), in an ingenious analysis not all of which is perfectly clear, comes to the opposite conclusion. It seems to the writer that such a case as that of the one here under discussion, in which there is an inversion in both arms of the chromosomes and in which only double cross-overs can occur, and, further, in which doubles may occur in both arms simultaneously, shows that synapsis need not necessarily begin either at the middle of the chromosome or at the ends. (Since, of course, neither of these regions (see Text-fig. 4) undergoes any synapsis at all.)

Crossing-over in the third chromosome.

Since no translocation exists in Plum between the second and third chromosomes, no alteration in the crossing-over relationships of the latter is to be expected. Tests were made, however, with the view of using the data obtained as controls for the mutual translocations studied. *V1/Cy* females were crossed to males which were (*Cy*) *Prple/Mo1*. "*Prple*" is a multiple stock containing the third chromosome characters roughoid (*ru*—0.0), hairy (*h*—26.5), thread (*th*—42.2), scarlet (*st*—44.0), ebony (*e*—70.7), and Prickly (*Pr*—90.0). The F_1 *V1/Prple* (*Cy*) females were back-crossed to *rucuca/DC_x* males (*rucuca* is a multiple stock containing the third chromosome characters *ru h th st cu sr e^s ca*)¹. The F_2 offspring showing Dichaete were discarded and the remainder classified. The results are given in Table II.

¹ *cu* is curled (50.0), *ca* is claret (100.7).

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It is to be seen that crossing-over in all regions except the short central one between thread and scarlet is higher than normal. Whether these higher values are due to the suppression of crossing-over in II at the same time, or whether other environmental factors entered in, remains uncertain. No plausible explanation can be given, either, for the lowered

TABLE II.

$$P_1: \frac{VI}{Cy} \text{♀♀} \times \frac{+ \text{ } ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr}{(Cy) \text{ } MoI} \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{VI}{(Cy)} \frac{+}{ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr} \text{♀♀} \times \frac{ru \text{ } h \text{ } th \text{ } st \text{ } cu \text{ } sr \text{ } e^s \text{ } ca}{DC_x} \text{♂♂}.$$

$$\frac{ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr}{\begin{array}{ccccc} 1 & 2 & 3 & 4 & 5 \end{array}}$$

(Cy and VI disregarded.)

0	<i>ru h th st e Pr</i>	192	3.4	<i>ru h th e Pr</i>	1
	+	336		<i>st</i>	1
1	<i>ru</i>	159	3.5	<i>ru h th Pr</i>	—
	<i>h th st e Pr</i>	125		<i>st e</i>	1
2	<i>ru h</i>	112	4.5	<i>ru h th st Pr</i>	25
	<i>th st e Pr</i>	101		<i>e</i>	30
3	<i>ru h th</i>	1	1.2.4	<i>ru th st</i>	8
	<i>st e Pr</i>	3		<i>h e Pr</i>	27
4	<i>ru h th st</i>	103	1.2.5	<i>ru th st e</i>	4
	<i>e Pr</i>	135		<i>h Pr</i>	6
5	<i>ru h th st e</i>	73	1.3.4	<i>ru st</i>	2
	<i>Pr</i>	122	1.3.5	<i>ru st e</i>	1
1.2	<i>ru th st e Pr</i>	14	1.4.5	<i>ru e</i>	10
	<i>h</i>	29		<i>h th st Pr</i>	12
1.4	<i>ru e Pr</i>	59	2.4.5	<i>ru h e</i>	6
	<i>h th st</i>	79		<i>th st Pr</i>	13
1.5	<i>ru Pr</i>	46	1.2.4.5	<i>ru th st Pr</i>	1
	<i>h th st e</i>	55		<i>h e</i>	2
2.4	<i>ru h e Pr</i>	45	1.3.4.5	<i>h th e</i>	1
	<i>th st</i>	58			
2.5	<i>ru h Pr</i>	33		Total	2070
	<i>th st e</i>	39			

Percentages of crossing-over.

Region	1	2	3	4	5
Test	31.0	24.1	0.5	29.8	23.1
Standard	26.5	15.7	1.8	26.7	19.3

crossing-over value for the *th—st* region; but it was found to be characteristic not only of this test with Plum, but also of all other tests made using *Prple* and the various mosaic eye colours.

TESTS INVOLVING DISCOLOURED (V2).

Discoloured females were crossed to *S/Cy D/C_{IIIx}* males, and the *F₁* *V2/Cy D* females were bred with normal males. Discoloured showed

random assortment with *Dichaete* in the following generation, proving that there was not present any translocation between II and III. Discoloured is not sex-linked; this shows there is no translocation between II and the X-chromosome.

Crossing-over in the second chromosome.

V2/Cy females were crossed to *Brisple/Cy* males; and the F_1 *V2/Brisple* females were back-crossed to *apl/Cy* males. The results obtained from this cross are given in Table III. Extensive tests were not made with Discoloured for the reason that, since the early work was done upon it by Muller (1930*b*), the stock has been complicated by the inclusion of several unknown factors affecting the eye colour; and until these can be studied in detail, the classification of results is exceedingly complex.

TABLE III.

$P_1: \frac{V2}{Cy} \text{♀♀} \times \frac{al\ dp\ b\ Bl\ c\ px\ sp}{Cy} \text{♂♂};$									
$F_1 \text{ back-cross: } \frac{al\ dp\ b\ Bl\ c\ px\ sp}{V2} \text{♀♀} \times \frac{al\ dp\ b\ pr\ c\ px\ sp}{Cy} \text{♂♂.}$									
	<i>al</i>	<i>dp</i>	<i>b</i>	<i>Bl</i>	<i>c</i>	<i>px</i>	<i>sp</i>		
	1	2	3	4	5	6	7		
	<hr/>								
	<i>V2</i>								
(Cy flies discarded.)									
0	<i>al dp b Bl c px sp</i>	77				1.2	<i>al b Bl c px sp</i>	1	
	<i>V2</i>	129					<i>dp V2</i>	2	
1	<i>al V2</i>	29				1.3	<i>al Bl c px sp</i>	—	
	<i>dp b Bl c px sp</i>	11					<i>dp b V2</i>	5	
2	<i>al dp V2</i>	60				2.3	<i>al dp Bl c px sp</i>	1	
	<i>b Bl c px sp</i>	70					<i>b V2</i>	8	
3	<i>al dp b V2</i>	17				3.4	<i>Bl V2</i>	1	
	<i>Bl c px sp</i>	8				4.5	<i>c V2</i>	1	
4	<i>al dp b Bl V2</i>	—				2.4.5	<i>al dp c V2</i>	3	
	<i>c px sp</i>	4							
							Total	427	
Percentages of crossing-over.									
Region	1	2	3	4	5	6	7		
Test	11.2	34.0	9.4	2.1	0.2	0.0	0.0		
Standard	13.0	35.5	6.2	20.8	25.0	4.0	2.5		

Although the data obtained were too scanty for thorough analysis, it can be seen from them that crossing-over in the left arm of II remains nearly normal; while in the right arm it occurs only in the form of a few single cross-overs between *Bl* and *c*, and a few doubles in the region of *c*. This is evidently best explained by the presence of an inversion in the

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right arm of II. One break probably occurred at the locus of brown, since evidence from the tests conducted on mutual translocations of this series of allelomorphs show that the eye character (brown colour and mosaicism) are inseparable from the break by crossing-over. No recombinations of *V2* and *sp* are found in the above table. The other break doubtless occurred between *Bl* and *c*.

Crossing-over in the third chromosome was not tested in the case of Discoloured.

TESTS INVOLVING TARNISHED (*V3*).

As in the preceding cases, Tarnished was tested for translocations between II and III, by crossing to *S/Cy D/C_{IIIx}*, and then back-crossing the *V3/Cy D* female offspring to normal males. In the following generation only *V3* and *Cy D* individuals were found. The absence of recombinations between *V3* and *D* is proof of the existence of a translocation between the second and the third chromosomes. It was also found that Tarnished males never appeared, all crosses having to be conducted with females. This was due to a sex-linked lethal (recessive), combined with a translocation between II and the *X*-chromosome, which appeared at the same time as the II—III translocation. Later it was possible to rid the Tarnished stock of the lethal, and obtain fully viable and fertile males.

Crossing-over in the second chromosome.

V3/Cy females were crossed to *Brisple/Cy* males containing also *DC_x* in one third chromosome. In the next generation females were selected which were *V3/Brisple DC_x*, and were back-crossed to *apl/Cy* males. The results obtained in the next generation are tabulated in Table IV.

As in the case of Discoloured, the *b-Bl* region shows an amount of crossing-over much higher than normal. This was probably due to culture conditions, the mothers being raised on rich yeast food. This is the region especially sensitive to variations in environmental conditions. This particular effect is apparently a characteristic of the *Brisple* stock, as it appears in all other tests in which it was used. It will also be observed that all the values for crossing-over from the left end toward the right are normal or greater as far as curved. Then crossing-over rapidly diminishes as the point of breakage is approached, this being the point marked by *Dichaete* among the second-chromosome characters. The point of breakage itself never separates either from the normal allelomorph of *sp* or from Tarnished, at the locus of *bw*. If not actually at the latter point, the break must certainly be very close to it. It is interesting to note that the

effect of the break on crossing-over is not confined to the point of break-age, but is transmitted with gradually diminishing effect to other regions, being still very marked in the region between *c* and *px*. But it does not extend across the spindle fibre.

TABLE IV.

$$P_1: V3/Cy \text{ } \varphi\varphi \times \frac{al \ dp \ b \ Bl \ c \ px \ sp}{Cy} \frac{DC_x}{DC_x} \text{ } \delta\delta;$$

$$F_1 \text{ back-cross: } \frac{V3}{al \ dp \ b \ Bl \ c \ px \ sp} \frac{DC_x}{DC_x} \text{ } \varphi\varphi \times \frac{al \ dp \ b \ pr \ c \ px \ sp}{Cy} \text{ } \delta\delta.$$

<i>al</i>	<i>dp</i>	<i>b</i>	<i>Bl</i>	<i>c</i>	<i>px</i>	<i>sp</i>
1	2	3	4	5	6	7
V3						

(Cy flies discarded.)

0	<i>al dp b Bl c px sp D</i>	52	2.3	<i>al dp Bl c px sp D</i>	1
	<i>V3</i>	253		<i>b V3</i>	19
1	<i>al V3</i>	59	2.4	<i>al dp c px sp D</i>	9
	<i>dp b Bl c px sp D</i>	4		<i>b Bl V3</i>	22
2	<i>al dp V3</i>	130	2.5	<i>al dp px sp D</i>	2
	<i>b Bl c px sp D</i>	80		<i>b Bl c V3</i>	2
3	<i>al dp b V3</i>	30	3.4	<i>al dp b c px sp D</i>	6
	<i>Bl c px sp D</i>	17		<i>Bl V3</i>	9
4	<i>al dp b Bl V3</i>	42	3.5	<i>al dp b px sp D</i>	—
	<i>c px sp D</i>	48		<i>Bl c V3</i>	5
5	<i>al dp b Bl c V3</i>	7	4.5	<i>al dp b Bl px sp D</i>	—
	<i>px sp D</i>	20		<i>c V3</i>	4
6	<i>al dp b Bl c px V3</i>	—	1.2.4	<i>al b Bl V3</i>	2
	<i>sp D</i>	1	1.2.5	<i>al b Bl c V3</i>	1
1.2	<i>al b Bl c px sp D</i>	4	1.3.4	<i>al Bl V3</i>	3
	<i>dp V3</i>	9		<i>dp b c px sp D</i>	3
1.3	<i>al Bl c px sp D</i>	3	2.3.4	<i>al dp Bl V3</i>	1
	<i>dp b V3</i>	6		<i>b c px sp D</i>	6
1.4	<i>al c px sp D</i>	8	3.4.5	<i>Bl px sp D</i>	2
	<i>dp b Bl V3</i>	6			
1.5	<i>al px sp D</i>	2		Total	880
	<i>dp b Bl c V3</i>	2			

Percentages of crossing-over.

Region	1	2	3	4	5	6	7
Test	12.7	32.7	12.6	19.4	5.7	0.11	0.0
Standard	13.0	35.5	6.2	20.8	25.0	4.0	2.5

Considerable variations in the sizes of some of the complementary classes are unfortunately present. These are due to the lower viability of flies carrying a number of recessive genes, together with the presence of *DC_x*, which is somewhat deleterious. Dumpy seemed also in these crosses to be especially harmful to the viability of flies carrying it.

Crossing-over in the third chromosome.

V3/Cy females were crossed to *Cy Prple/Mo1* males. The F_1 females which were *Cy Prple/V3* were back-crossed to *rucuca/DC_x* males. The results of classifying the non-*D* offspring are presented in Table V.

TABLE V.

$P_1: \frac{V3}{Cy} \text{ } \varnothing\varnothing \times \frac{Cy \text{ } ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr}{+ \text{ } MoI} \text{ } \delta\delta;$																																							
$F_1 \text{ back-cross: } \frac{V3}{Cy \text{ } ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr} \text{ } \varnothing\varnothing \times \frac{ru \text{ } h \text{ } th \text{ } st \text{ } cu \text{ } sr \text{ } e^s \text{ } ca}{DC_x} \text{ } \delta\delta.$																																							
<table> <tr> <td></td> <td><i>ru</i></td> <td><i>h</i></td> <td><i>th</i></td> <td><i>st</i></td> <td></td> <td><i>e</i></td> <td><i>Pr</i></td> <td></td> <td></td> </tr> <tr> <td></td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> <td></td> <td>5</td> <td>6</td> <td></td> <td></td> </tr> <tr> <td></td> <td colspan="8"><hr/></td> <td><i>V3</i></td> </tr> </table>											<i>ru</i>	<i>h</i>	<i>th</i>	<i>st</i>		<i>e</i>	<i>Pr</i>				1	2	3	4		5	6				<hr/>								<i>V3</i>
	<i>ru</i>	<i>h</i>	<i>th</i>	<i>st</i>		<i>e</i>	<i>Pr</i>																																
	1	2	3	4		5	6																																
	<hr/>								<i>V3</i>																														
(Dichaete flies discarded.)																																							
0	<i>ru h th st e Pr Cy</i>	347				1.5	<i>ru V3 e Pr</i>		3																														
	<i>V3</i>	554					<i>h th st Cy</i>		9																														
1	<i>ru V3</i>	20				1.6	<i>ru V3 Pr</i>		12																														
	<i>h th st e Pr Cy</i>	12					<i>h th st e Cy</i>		8																														
2	<i>ru h V3</i>	10				2.5	<i>ru h V3 e Pr</i>		6																														
	<i>th st e Pr Cy</i>	7					<i>th st Cy</i>		6																														
3	<i>ru h th V3</i>	—				2.6	<i>ru h V3 Pr</i>		4																														
	<i>st e Pr Cy</i>	—					<i>th st e Cy</i>		4																														
4	<i>ru h th st V3</i>	1				5.6	<i>ru h th st Pr Cy</i>		34																														
	<i>e Pr Cy</i>	1					<i>V3 e</i>		49																														
5	<i>ru h th st Cy</i>	185				1.2.5	<i>ru th st Cy</i>		1																														
	<i>V3 e Pr</i>	256				1.2.6	<i>ru th st e Cy</i>		1																														
6	<i>ru h th st e Cy</i>	134				1.5.6	<i>h th st Pr Cy</i>		1																														
	<i>V3 Pr</i>	192				3.5.6	<i>st Pr Cy</i>		1																														
1.2	<i>ru th st e Pr Cy</i>	5				1.2.4.5	<i>h Cy</i>		1																														
	<i>h V3</i>	1							1																														
1.4	<i>ru e Pr Cy</i>	1						Total	1866																														
	<i>h th st V3</i>	—																																					

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test	4.0	2.5	0.05	0.22	29.6	23.6
Standard	26.5	15.7	1.8		26.7	19.3
Control	31.0	24.1	0.5		29.8	23.1

As in the case of Plum, the crossing-over values for the *th-sl* region are too small; this is probably due to the particular stocks used, and has no significance for the purposes of the experiment. We may consider the data found for Plum as controls in the present case (see Table II). With this in mind, we find that crossing-over in the right arm of III in Tarnished is normal in amount, but that for the left arm it is significantly lower than normal. This cannot be due to an inversion, it would seem, since the cross-overs which do occur in this arm are *single* cross-overs.

It is then probable, although the point has not yet been tested, that this is the region involved in the translocation to the *X*-chromosome; and the extraordinarily low amount of crossing-over between thread and scarlet leads to the belief that the break is in that region.

To determine more accurately the locus of the break connected with *V3*, with respect to the neighbouring loci *st* and *cu*, *V3/Cy* females were crossed to *rucuca/DC_x* males, and the *F*₁ heterozygous *V3/rucuca* females were back-crossed to *rucuca/DC_x* males. The classification of the non-*D* *F*₂ offspring was only carried out with respect to the loci *st*, *V3*, *cu*, and *e*, and is given in Table VI.

TABLE VI.

$$P_1: \frac{V3}{Cy} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{DC_x} \text{ } \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V3}{+} \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{+} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{DC_x} \text{ } \text{♂♂}.$$

<i>st</i>	<i>cu</i>	<i>e</i>
1	2	3
<i>V3</i>		

(Dichaete flies discarded; only loci *st*, *V3*, *cu*, and *e* studied.)

0	<i>st cu e</i>	255	1.2	<i>st V3 cu e</i>	—
	<i>V3</i>	335		+	2
1	<i>st V3</i>	1	1.3	<i>st V3 e</i>	1
	<i>cu e</i>	7		<i>cu</i>	—
2	<i>st</i>	15	2.3	<i>st e</i>	2
	<i>V3 cu e</i>	32		<i>V3 cu</i>	3
3	<i>st cu</i>	87	1.2.3	<i>st V3 cu</i>	—
	<i>V3 e</i>	123		<i>e</i>	1
					Total
					864

Percentages of crossing-over.

Region	1	2	3
Test	1.4	6.4	25.1
Standard		6.0	20.7

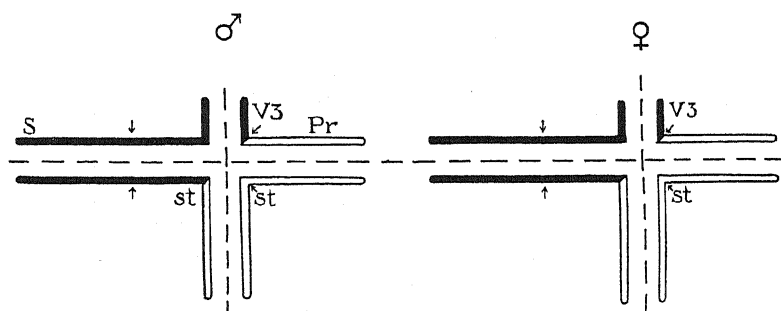
These data locate Tarnished at about 1.1 units to the left of *st*, or at approximately locus 45.4. This being to the left of the spindle-fibre attachment, we expect III *L* to be attached to II *L*, which has a spindle fibre, and hence also II *R* to III *R* (where *L* and *R* designate the pieces to the left and right of the breaks respectively). In the next section this hypothesis is demonstrated to be correct.

*Determination of the actual connections between
the broken pieces in Tarnished.*

Next a test was devised which would furnish information as to the actual connections between the broken pieces of II and III in Tarnished.

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The small right-end piece of II is conveniently marked by the dominant brown allelomorph, in this case *V3*. By crossing-over, the dominant Prickly (*Pr*), at 90.0 in the right end of III, and scarlet, at 44.0, just to the left of the spindle-fibre attachment of III, were inserted into the affected chromosomes to act as markers. Males carrying these three genes were then crossed to females bearing the dominant Star (*S*), at 1.3 in II, and the recessive scarlet. Male offspring from this cross which were *S V3 st Pr* were then crossed to females carrying the translocation *V3* and containing *st* heterozygous, in their normal III. This is shown in the diagram below (Text-fig. 5), where homologous parts are shown in apposition. Chromosome II or parts derived from it are shown in solid



Text-fig. 5.

lines, III and parts derived from it with double lines; spindle-fibre attachment points are marked with arrows. In this diagram, the connection of parts below shown to obtain is here already assumed as true.

In this cross, individuals arising from gametes having the "orthoploid"¹ combinations of genes (those placed diagonally apart from one another in the diagram) will be phenotypically *V3 st Pr*, *SV3* and *S st* respectively, the fourth class (*V3/V3 Pr*) being almost completely absent owing to the fact that Tarnished is nearly 100 per cent. lethal in the homozygous condition. It is evident that in the formation of these classes all homologous elements underwent disjunction. These should hence be the most frequent classes, as shown by Muller (1930c) for *Drosophila* in his study of the "Star Curly" translocation. The "aneuploid"² gametes will give rise to non-viable zygotes except when each is

¹ We use the word "orthoploid" to refer to gametes which possess a complete set of genes for both chromosomes. In the present case these happen to be the same as the paternal and maternal combinations.

² We use the word "aneuploid" to refer to gametes possessing deficiencies of some chromosome portions and/or duplications of other portions.

matched by the complementary class from the other parent. If the connections of the translocated pieces are, as shown above, II *L* to III *L* and II *R* to III *R*, the moderately aneuploid gametes, formed when the major portions of II and III (that is, II *L* and III *R*) segregate from each other and only the smaller portions undergo non-disjunction, will form individuals phenotypically *S V3 Pr* and *V3 st*. (In this case, the separation of chromosomes would have taken place as shown by the horizontal line dividing them in the diagram above.) We should expect that these moderately aneuploid gametes would be formed in greater numbers than the extremely aneuploid gametes which involve non-disjunction of a larger amount of chromatin; at least, if there is any large difference in the frequency between the two types of aneuploid classes, the moderately aneuploid should be the more numerous, and the extremely aneuploid classes the less numerous. (The latter classes would, in the above figure, result from separation to the sides of the vertical lines between the chromosomes.) They would produce zygotes phenotypically *V3 Pr* and *S V3 st*, and hence, in case large differences in the frequency of aneuploid classes did occur, these phenotypes should be less numerous than the *S V3 Pr* and *V3 st* phenotypes, provided the above assumed chromosomal connections really exist. On the other hand, if the connections had been II *L* to III *R* and II *R* to III *L*, the classes from the extremely aneuploid gametes would have been, contrariwise, *S V3 Pr* and *V3 st*, i.e. the relations and frequencies of the classes in question would have been reversed. In the case of Tarnished a glance at the data (Table VII) shows that the latter classes (*S V3 Pr* and *V3 st*) are far more numerous than the former classes. This indicates that the first assumed chromosomal connections (those shown in the diagram) are those which really exist.

TABLE VII.

S V3 Pr st/st ♂♂ × *V3 st/+* ♀♀.

(See Text-fig. 5.)

<i>V3 st Pr</i>	245	Orthoploid
<i>S V3</i>	187	
<i>S st</i>	211	
<i>S V3 Pr</i>	81	Moderately aneuploid
<i>V3 st</i>	132	
<i>V3 Pr</i>	12	Others (including homozygous <i>V3/V3 Pr</i> , cross-overs, and extremely aneuploid individuals, if any)
<i>S V3 st</i>	3	
<i>S V3 st Pr</i>	3	
Total	874	

But although this test reveals clearly the actual connections which exist between the broken pieces of II and III (namely, II *L* to III *L* and II *R* to III *R*), it does not with equal certainty prove the actual occurrence of the extremely aneuploid class of gametes. In order to do this, the test must be so formulated that no crossing-over between *st* and *V3* can occur in the female parent. This is necessary because the occurrence of such rare cross-overs, to be expected in the case of Tarnished (see Table V) in about 0.22 per cent., would give rise to flies identical in appearance with the extremely aneuploid classes. This can be readily seen from the diagram (Text-fig. 5). The test was therefore rearranged so that the uncertainty is avoided. *V3 st Pr* males from the preceding cross were crossed with *S/Cy D/C_{IIIx}* females, and *V3 st* males from the same cross were crossed to *st* females. Then, from the offspring of these two crosses, *Cy V3 Pr* males (which must also have carried *st* heterozygous and, as well, the *C_{IIIx}* chromosome) were crossed with females which were *V3 st*. This cross is in progress at the time of writing.

The ratios between the zygotes formed from the three meiotic classes, allowing for the fact that one class from the orthoploid gametes is very inviable (the few individuals hatching would be *V3 Pr* in phenotype, and form the majority of the twelve individuals so listed in the table) are of the order 2:1 (the small number of extremely aneuploid individuals may be neglected in this comparison). The gametes will have been formed in the ratio which is the square root of this (as shown by Muller, 1930c), namely, 1.4:1. This is smaller than the ratio of 1.7 found by Muller for a presumably simple translocation, a fact which might be explained by assuming the breakage of III to have been nearer the physical mid-point in his case¹.

Dobzhansky and Sturtevant have recently published a valuable account of mutual translocations between the second and third chromosomes of *Drosophila* (1931). In their translocation *B*, in which the second chromosome is broken at some distance from the cytological middle, between *b* and *pr* in the left arm, and the third chromosome is broken near the middle, they have a case which is similar to Tarnished. Their data indicate that only four types of gametes are formed by this translocation.

It may be pointed out that the method described above, in which each portion (right and left of all the possible breaks) of each chromosome is

¹ In a conversation with Dr Dobzhansky, he pointed out the fact that obviously this calculation is based on the assumption that the respective types of gametes are formed in equal proportions in male and female parents. Although probable, it is not absolutely certain that this is actually the case.

differentially marked from the homologous portions of both parents, is the only genetic method by which mutual translocations may be distinguished from non-mutual with certainty. It is of particular importance that the small pieces, if there are any such, should be so marked. The fact that such tests have not until the present been tried with most translocations leads to the belief that most, if not all, translocations are mutual in nature. Since the above was written, Burnham has published an account of certain similar types of translocations in *Zea mays*, the study of which leads him also to the conclusion that "it is probable that some of the types suspected of being simple translocations in *Drosophila* would prove to be interchanges if critical cytological evidence could be obtained" (Burnham, 1932). But critical cytological evidence is very difficult to obtain in *Drosophila*, so that the present type of genetic test is more favourable.

The method described above should be of still further value, in that through it an analysis of the position of the spindle fibre with reference to known genes can be made. When one chromosome is broken near the middle, and the other at some point more or less remote from the middle, as is the case in Tarnished, a cross of two individuals each heterozygous for the translocation, one of which contains a given marker close to the spindle fibre, and the other of which does not, will allow a determination to be made as to whether the spindle-fibre attachment is to the right or the left of this marker, provided the break is between the locus of the marker and that of the spindle fibre. Thus, from the above test, in which the male parent contained the gene for scarlet in the affected chromosomes, while the female did not, and the marker *Pr* was present in the same chromosome, the point of the spindle-fibre attachment of III can be determined with reference to *st*. We know the location of the spindle-fibre attachment in the said case remains with III *R*, since II *R* has no spindle fibre and is attached to it (Text-fig. 5). Then, since in the individuals arising from the moderately aneuploid gametes, *st* separates from *Pr*, which is in the right arm of III, the spindle fibre must be to the right of *st*. This, of course, from the triploid method of Redfield (1930), we already recognised to be the case. From cytological evidence, Dobzhansky (1930 *b*) has judged that the spindle-fibre attachment lies between 46.0 and 48.0. If then, instead of *st* we use some other marker closer to the probable location, such as the dominant Deformed eye (*Df*), at 47.5, and if, in the moderately aneuploid individuals, *Df* separates from *Pr*, the spindle fibre must be attached to the right of *Df*. But if *Df* remains with *Pr*, we merely ascertain that *Df* is to the right of the break, which may or

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may not be to the right of the spindle fibre. Tests of our present kind with several such translocations, using various markers, would result in still greater refinement in the location of the spindle fibre. Since the middle region, just to right and left of the spindle fibre, is known to be relatively crowded with genes, and since crossing-over, by which means we must seek to get the desired markers into the chromosomes carrying the translocation, is in this very region most susceptible to environmental agencies, we may hope to determine the position of the spindle fibre with great exactitude. The method can of course be applied equally readily to the second chromosome, or to similar cases in other forms.

TESTS INVOLVING ROSY (*V4*).

V4/Cy females were crossed to *S/Cy D/C_{IIIx}* males, and the F_1 *V4/Cy D* males were then crossed to normal females. In the F_2 generation there was no recombination between *V4* and *D*, a proof of the existence of a translocation between II and III.

Crossing-over in the second chromosome.

As in the preceding cases, *V4* was tested for crossing-over in II by crossing to *Brisple/Cy*, and back-crossing to *apl/Cy*. The results are given in Table VIII. As in the case of Plum, column 1 represents crossing-over when crossing-over in III was not hindered; column 2 presents the data obtained when it was checked by the presence of *DC_x*. In the *Brisple* stock used in the first instance black was not present¹.

It will be seen from this table that the percentages for the second column are higher than those in the first, a result which confirms Dobzhansky and Sturtevant's (1931) finding that in mutual translocations suppression of crossing-over in one of the concerned chromosomes increases it in the other, at least in its affected arm. The presence of *Dichaete* in the second test is of additional value in that it affords a marker for the position of the break in II. This is so closely linked with both *V4* itself and with speck that no crossing-over occurs between them. We cannot assume it proved that the break lies exactly at either of these two loci, however, since crossing-over is negligible through the whole *px-sp* region. Indeed, some of the few individuals apparently resulting from crossing-over between *px* and the break (*al dp b Bl c sp D* and *al dp sp D*) may be due to the difficulty of distinguishing *px* when *dp* is present. These flies were unfortunately of very low viability, and the attempt to breed them was unsuccessful. It should be further observed

¹ Parentheses are used to indicate that a character is present in only a part of the cases.

that Rosy and Dichaete separate in segregation 100 per cent. of the time (three apparent exceptions were observed, but were proved to have been

TABLE VIII.

$$P_1: \frac{V4}{Cy} \text{♀♀} \times \frac{al dp(b)Bl c px sp (DC_x)}{Cy} \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V4}{al dp(b)Bl c px sp (DC_x)} + \text{♀♀} \times \frac{al dp(b)Bl c px sp}{Cy} \text{♂♂}.$$

<i>al</i>	<i>dp</i>	<i>b</i>	<i>Bl</i>	<i>c</i>	<i>px</i>	<i>sp</i>
1	2	3	4	5	6	7

V4

(Cy flies discarded.)

		1	2			1	2
0	<i>al dp(b)Bl c px sp(D)</i>	153	55	3.4	<i>al dp(b)c px sp(D)</i>	26	7
	V4	230	523		Bl V4	39	15
1	<i>al V4</i>	21	71	4.5	<i>al dp(b)Bl px sp(D)</i>	—	—
	<i>dp(b)Bl c px sp(D)</i>	21	6		c V4	1	6
2	<i>al dp V4</i>	136	148	4.6	<i>al dp(b)Bl sp(D)</i>	—	—
	<i>(b)Bl c px sp(D)</i>	113	127		c px V4	1	—
3	<i>al dp(b)V4</i>	(Incl.	46	3.5	<i>al dp(b)px sp(D)</i>	9	2
	<i>Bl c px sp(D)</i>	in 2)	34		Bl c V4	5	5
4	<i>al dp(b)Bl V4</i>	19	47	1.2.3	<i>dp Bl c px sp(D)</i>	—	1
	<i>c px sp(D)</i>	47	119	1.2.4	<i>al(b)Bl V4</i>	—	4
5	<i>al dp(b)Bl c V4</i>	8	9		<i>dp c px sp(D)</i>	1	1
	<i>px sp(D)</i>	17	35	1.2.5	<i>al(b)Bl c V4</i>	—	1
1.2	<i>al(b)Bl c px sp(D)</i>	8	8	1.3.4	<i>al Bl V4</i>	2	3
	<i>dp V4</i>	6	24		<i>dp(b)c px sp(D)</i>	—	2
1.3	<i>al Bl c px sp(D)</i>	(Incl.	3	1.3.5	<i>dp(b)px sp(D)</i>	1	1
	<i>dp(b)V4</i>	in 1.2)	12	1.4.5	<i>al c V4</i>	—	4
1.4	<i>al c px sp(D)</i>	2	28	2.3.4	<i>al dp Bl V4</i>	(Incl.	7
	<i>dp(b)Bl V4</i>	1	12		<i>(b)c px sp(D)</i>	in 4)	6
1.5	<i>al px sp(D)</i>	4	2	2.3.5	<i>(b)px sp(D)</i>	(Incl.	5
	<i>dp(b)Bl c V4</i>	—	4			in 5)	
2.3	<i>al dp Bl c px sp(D)</i>	(Incl.	6	2.4.5	<i>al dp c V4</i>	2	1
	<i>(b)V4</i>	in 0)	27		<i>(b)Bl px sp(D)</i>	—	1
2.4	<i>al dp c px sp(D)</i>	(Incl.	27	1.2.3.5	<i>al(b)px sp(D)</i>	—	1
	<i>(b)Bl V4</i>	in 3.4)	49	5.6	<i>al dp(b)Bl c sp(D)</i>	—	1
2.5	<i>al dp px sp(D)</i>	(Incl.	4		<i>px V4</i>	—	—
	<i>(b)Bl c V4</i>	in 3.5)	8				
2.6	<i>al dp sp(D)</i>	—	1		Total	873	1509
	<i>(b)Bl c px V4</i>	—	—				

Percentages of crossing-over.

Region	1	2	3	4	5	6	7
Test (1)	7.9	39.9		16.2	5.4	0.3	0.0
(2)	12.5	30.3	11.7	22.4	6.0	0.13	0.0
Standard	13.0	35.5	6.2	20.8	25.0	4.0	2.5

due to occasional crossing-over in the DC_x chromosome). This is an indication that the break is very close to the gene concerned, which we have

from previous evidence shown to be brown; and is similar to the findings for Tarnished previously discussed. Again, as in Tarnished, it is to be observed that the effect of the break in diminishing crossing-over extends markedly over into the *c-pr* region, and somewhat into the *Bl-c* region, being therefore not confined to its immediate vicinity, although it is greatest there. When, however, crossing-over is prevented in III, the effect is not observed to extend so far as to the *Bl-c* region, even though this is on the same side of the spindle fibre as the break.

Crossing-over in the third chromosome.

V4/Cy females were crossed to *(Cy)Prple/Mo1* males, and the F_1 female offspring which were *V4/(Cy)Prple* were back-crossed to *rucuca/DC₂* males. The results are given in Table IX, column 1 giving the data when *Cy* was not present in the female parent, column 2 when it was.

It is again apparent that crossing-over is much greater in III when simultaneously suppressed in II than when it is not suppressed in II. This is further confirmation of Dobzhansky and Sturtevant (1931). The *e-Pr* region forms an exception which remains unexplained; but it is far from the region of disturbance and the observed difference is slight. The low crossing-over value for the *th-st* region characteristic of these crosses is again seen. There were two exceptions to the complete separation of *Cy* and *V4* in the F_2 offspring which are not shown on the table; one *V4 Cy* individual, and one *ru Pr V4 Cy*. These were found to be due to crossing-over between *Cy* and *V4* in the balanced *V4/Cy* stock from which parents were originally taken. Again, as in the case of Tarnished, we find that, when crossing-over is prevented in II, crossing-over in both arms of III is normal or above in amount. Evidently, when the break in an autosome occurs near the spindle-fibre attachment, crossing-over is not markedly affected by it. This is in agreement with the findings of Dobzhansky (1932) for II to Y translocations where II was broken in the middle.

To determine the effects of the break and reattachment upon the *st-e* region more exactly, *V4/Cy* females were crossed to *rucuca* males; and the F_1 *V4/rucuca* females were back-crossed to *rucuca* males. The results in F_2 for the loci *st*, *cu*, *e*, and *V4* are presented in Table X.

The data from this table place the break in III of *V4* at a point about 2.7 units to the right of *st*, at approximately locus 46.7, a result in agreement with the percentages given in Table IX. This is probably to the left of the spindle fibre, and leads us to conjecture that the connections of

the broken pieces are II *L* to III *L* and II *R* to III *R*. In the next section this is demonstrated to be in fact the case.

TABLE IX.

$P_1: \frac{V4}{Cy} \text{♀♀} \times \frac{(Cy) \text{ } ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr}{+ \text{ } MoI} \text{♂♂};$							
$F_1 \text{ back-cross: } \frac{V4}{(Cy) \text{ } ru \text{ } h \text{ } th \text{ } st \text{ } e \text{ } Pr} \text{♀♀} \times \frac{ru \text{ } h \text{ } th \text{ } st \text{ } cu \text{ } sr \text{ } e^s \text{ } ca}{DC_x} \text{♂♂}.$							
		$\begin{array}{cccccc} ru & h & th & st & e & Pr \\ \hline 1 & 2 & 3 & 4 & 5 & 6 \\ \hline V4 \end{array}$					
		(Dichaete flies discarded.)					
		1	2			1	2
0	<i>ru h th st e Pr(Cy)</i>	88	148	3.4	<i>st V4</i>	—	1
	<i>V4</i>	137	317	3.5	<i>st(Cy)</i>	—	1
1	<i>ru V4</i>	23	111	3.6	<i>st e(Cy)</i>	—	1
	<i>h th st e Pr(Cy)</i>	24	89	4.5	<i>(Cy)</i>	1	4
2	<i>ru h V4</i>	20	74	4.6	<i>ru h th st V4 Pr</i>	—	1
	<i>h th st e Pr(Cy)</i>	24	74		<i>e(Cy)</i>	1	8
3	<i>st e Pr(Cy)</i>	—	4	5.6	<i>ru h th st Pr(Cy)</i>	—	12
4	<i>ru h th st V4</i>	—	9		<i>V4 e</i>	1	22
	<i>e Pr(Cy)</i>	1	12	1.2.5	<i>ru th st(Cy)</i>	1	2
5	<i>ru h th st(Cy)</i>	21	75		<i>h V4 e Pr</i>	1	2
	<i>V4 e Pr</i>	21	126	1.2.6	<i>ru th st e(Cy)</i>	—	3
6	<i>ru h th st e(Cy)</i>	28	36		<i>h V4 Pr</i>	1	4
	<i>V4 Pr</i>	36	95	1.4.5	<i>ru(Cy)</i>	—	3
1.2	<i>ru th st e Pr(Cy)</i>	1	16		<i>h th st V4 e Pr</i>	—	2
	<i>h V4</i>	2	24	1.5.6	<i>ru V4 e</i>	2	6
1.4	<i>ru e Pr(Cy)</i>	—	2		<i>h th st Pr(Cy)</i>	—	8
	<i>h th st V4</i>	—	3	2.3.4	<i>ru h st V4</i>	1	—
1.5	<i>ru V4 e Pr</i>	8	54	2.4.5	<i>th st V4 e Pr</i>	—	1
	<i>h th st(Cy)</i>	9	47	2.5.6	<i>ru h V4 e</i>	—	4
1.6	<i>ru V4 Pr</i>	15	35		<i>th st Pr(Cy)</i>	—	5
	<i>h th st e(Cy)</i>	11	28	4.5.6	<i>Pr(Cy)</i>	1	1
2.4	<i>th st V4</i>	1	1	1.2.5.6	<i>ru th st Pr(Cy)</i>	—	3
2.5	<i>ru h V4 e Pr</i>	1	30	1.4.5.6	<i>h th st V4 e</i>	—	1
	<i>th st(Cy)</i>	3	26				
2.6	<i>ru h V4 Pr</i>	13	18				
	<i>th st e(Cy)</i>	5	15				
						Total	502 1564

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test (1)	19.5	14.7	0.2	1.2	13.9	22.8
(2)	28.3	19.3	0.45	3.1	27.7	19.5
Control	31.0	23.1	0.5		29.8	23.1
Standard	26.5	15.7	1.8		26.7	19.3

Connections of the broken pieces in *V4*.

Tests similar to those carried out for the same purpose with Tarnished were made with Rosy also. Males which were *S st/V4 st Pr* were crossed to females which were *V4/st*. The results are given in Table XI.

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From these data it appears that Tarnished and *V4* are similar, in that in each the connections of the broken pieces are II *L* to III *L* and II *R* to III *R*. The large number of individuals in the *V4 Pr* class is due to the inclusion in it of individuals which are homozygous for *V4*, these appearing in larger numbers than usual, doubtless on account of unusually

TABLE X.

$$P_1: \frac{V4}{Cy} \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V4}{+} \dots \frac{+}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{♂♂}.$$

<i>st</i>	<i>cu</i>	<i>e</i>
1	2	3
<hr style="width: 100%; border: 0.5px solid black;"/>		
<i>V4</i>		

(Only loci of *st*, *cu*, *e* and *V4* observed.)

0	<i>st cu e</i>	285	3	<i>st cu</i>	79
	<i>V4</i>	528		<i>V4 e</i>	138
1	<i>st V4</i>	6	1.3	<i>st V4 e</i>	1
	<i>cu e</i>	7		<i>cu</i>	1
2	<i>st</i>	7	2.3	<i>st e</i>	2
	<i>V4 cu e</i>	8		<i>V4 cu</i>	1
Total					1063

Percentages of crossing-over.

Region	1	2	3
Test	1.4	1.7	20.8
Standard		6.0	20.7

TABLE XI.

$$P_1: S\ st/V4\ st\ Pr\ \text{♂♂} \times V4/st\ \text{♀♀}.$$

<i>V4 st Pr</i>	551	Orthoploid
<i>S V4</i>	526	
<i>S st</i>	509	
<i>S V4 Pr</i>	273	Moderately aneuploid
<i>V4 st</i>	277	
<i>V4 Pr</i>	49	Others (including extremely
<i>S V4 st</i>	7	aneuploid, homozygous <i>V4</i>
<i>S V4 st Pr</i>	2	and cross-over individuals)
Total	2194	

favourable environmental conditions. The two individuals which are *S V4 st Pr* must either have come from an extraordinary segregation in which three chromosomes went to one pole and one to the other, so that a gamete which was *S V4 st Pr* (3 chromosomes) united with one which was simply *st* (1 chromosome)—see Text-fig. 5; or, alternatively, the apparent Star was not really Star, but a few of the original parents con-

tained *ru*. These individuals were not tested to see whether or not this was the case; since the cross was to be repeated, as in the case of Tarnished, with males which were $V4\ st\ Pr/Cy$ crossed to females which were $V4\ st/st$. This test is being carried on at present.

The ratio between the zygotes of the two main meiotic classes is 1.9:1; and the ratio of the orthoploid gametes to the aneuploid is therefore 1.4:1. This is like the results obtained with Tarnished.

TESTS INVOLVING $V5$.

$V5/Cy$ females were crossed to $S/Cy\ D/C_{IIIx}$ males, and the F_2 progeny examined. None of the $V5$ offspring were D , showing that there was present a translocation involving II and III.

Crossing-over in the second chromosome.

$V5/Cy$ females were crossed to $Brisple/Cy(DC_x)$ males, and the female offspring which were $V5/Brisple(DC_x)$ were back-crossed to apl/Cy males. The F_2 offspring were classified, and the results are given in Table XII. In column 1 are presented the results when DC_x was not present, in column 2 those obtained when it was present. Differences between the two columns therefore represent differences in crossing-over in II when crossing-over in III is not simultaneously checked, and when it is, respectively. The *Brisple* stock used in the tests tabulated in column 1 did not contain black; therefore in this column regions 2 and 3 were not followed separately. The results indicate this stock had a considerably higher viability than the *Brisple* used for the tests given in column 2; this may somewhat affect our comparison of linkage values.

The increase in crossing-over when III is simultaneously inhibited from crossing-over does not this time seem to be significant in the left arm of II. It must be remembered that where the $dp-b$ and $b-Bl$ regions are lumped together, in column 1, the figure will be lower on account of the undetected double cross-overs in this region. If we allow for this, the difference between the two columns is too slight to be significant. But the extraordinary increase on the right side of the spindle fibre, in the $Bl-c$ region especially, is impressively large. It will again, as in the preceding cases, be noted that the effect of the break and reattachment upon crossing-over is extended from the locus of the break to some distance in the adjacent regions, but does not extend across the spindle-fibre attachment, into the other arm. And when crossing-over in III is suppressed, the influence does not even extend appreciably into the $Bl-c$ region.

TABLE XII.

$$P_1: \frac{V5}{Cy} \text{♀♀} \times \frac{al\ dp(b)Bl\ c\ px\ sp}{Cy} (DC_x) \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V5}{al\ dp(b)Bl\ c\ px\ sp\ (DC_x)} \text{♀♀} \times \frac{al\ dp\ b\ pr\ c\ px\ sp}{Cy} \text{♂♂}.$$

$\frac{al}{1}$	$\frac{dp}{2}$	$\frac{b}{3}$	$\frac{Bl}{4}$	$\frac{c}{5}$	$\frac{px}{6}$	$\frac{sp}{7}$
$V5$						

(Curly flies disregarded.)

		1	2			1	2
0	<i>al dp(b)Bl c px sp(D)</i>	259	61	3.5	<i>al dp(b)px sp(D)</i>	2	—
	<i>V5</i>	371	709		<i>Bl c V5</i>	3	3
1	<i>al V5</i>	68	100	3.6	<i>al dp(b)sp(D)</i>	1	—
	<i>dp(b)Bl c px sp(D)</i>	38	20	4.5	<i>al dp(b)Bl px sp(D)</i>	1	—
2	<i>al dp V5</i>	196	216		<i>c V5</i>	—	8
	<i>(b)Bl c px sp(D)</i>	181	121	5.6	<i>al dp(b)Bl c sp(D)</i>	—	1
3	<i>al dp(b)V5</i>	(Incl.	63	1.2.3	<i>al(b)V5</i>	—	1
	<i>Bl c px sp(D)</i>	in 2)	44	1.2.4	<i>al(b)Bl V5</i>	(Incl. in 4	4
4	<i>al dp(b)Bl V5</i>	17	62		<i>dp c px sp(D)</i>	1.3.4)	—
	<i>c px sp(D)</i>	33	118	1.2.5	<i>dp px sp(D)</i>	2	—
5	<i>al dp(b)Bl c V5</i>	5	6	1.3.4	<i>al Bl V5</i>	—	2
	<i>px sp(D)</i>	11	23		<i>dp(b)c px sp(D)</i>	3	1
6	<i>sp(D)</i>	—	1	1.4.5	<i>al c V5</i>	—	1
1.2	<i>al(b)Bl c px sp(D)</i>	1	4	2.3.4	<i>al dp Bl V5</i>	(Incl. 5	—
	<i>dp V5</i>	9	24		<i>(b)c px sp(D)</i>	in 4)	4
1.3	<i>al Bl c px sp(D)</i>	(Incl.	5	2.4.5	<i>al dp c V5</i>	—	1
	<i>dp(b)V5</i>	in 1.2)	12		<i>(b)Bl px sp(D)</i>	—	1
1.4	<i>al c px sp(D)</i>	3	10	2.4.6	<i>(b)Bl sp(D)</i>	1	—
	<i>dp(b)Bl V5</i>	5	7	3.4.5	<i>al dp(b)c V5</i>	—	1
1.5	<i>al px sp(D)</i>	1	4	1.2.3.4	<i>dp Bl V5</i>	(Incl. 1	—
	<i>dp(b)Bl c V5</i>	—	1			in 1.4)	—
2.3	<i>al dp Bl c px sp(D)</i>	(Incl.	9	1.2.4.5	<i>dp c V5</i>	—	1
	<i>(b)V5</i>	in 0)	30	2.3.4.5	<i>(b)c V5</i>	—	1
2.4	<i>al dp c px sp(D)</i>	(Incl.	33				
	<i>(b)Bl V5</i>	in 3.4)	41				
2.5	<i>al dp px sp(D)</i>	(Incl.	2				
	<i>(b)Bl c V5</i>	in 3.5)	5				
3.4	<i>al dp(b)c px sp(D)</i>	24	6				
	<i>Bl V5</i>	12	38				
					Total	1247	1815

Percentages of crossing-over.

Region	1	2	3	4	5	6	7
Test (1)	10.4		34.8	7.9	2.1	0.2	0.0
(2)	11.1	28.0	12.4	19.2	3.3	0.1	0.0
Standard	13.0	35.5	6.2	20.8	25.0	4.0	2.5

Crossing-over in the third chromosome.

V5/Cy females were crossed to *(Cy)Prple/Mo1* males, and the females which were *(Cy)Prple/V5* were selected from the offspring and mated to *rucuca/DC_x* males. The results obtained in the *F*₂ generation are given in

Table XIII, column 1 giving the results when *Cy* was not present; column 2 when it was present.

TABLE XIII.

$$P_1: \frac{V5}{Cy} \text{ } \varphi\varphi \times \frac{ru\ h\ th\ st\ e\ Pr}{(Cy)\ MoI} \text{ } \delta\delta;$$

$$F_1 \text{ back-cross: } \frac{V5}{(Cy)\ ru\ h\ th\ st\ e\ Pr} \text{ } \varphi\varphi \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{DC_x} \text{ } \delta\delta.$$

<i>ru</i>	<i>h</i>	<i>th</i>	<i>st</i>	<i>e</i>	<i>Pr</i>
1	2	3	4	5	6
V5					

(Dichaete flies disregarded.)

		1	2			1	2
0	<i>ru h th st e Pr(Cy)</i>	122	187	2.6	<i>ru h V5 Pr</i>	6	20
	<i>V5</i>	161	280		<i>th st e(Cy)</i>	5	19
1	<i>ru V5</i>	54	136	3.4	<i>ru h th V5 e Pr</i>	1	—
	<i>h th st e Pr(Cy)</i>	35	81	3.6	<i>st e(Cy)</i>	1	—
2	<i>ru h V5</i>	21	55	4.5	<i>ru h th st V5 e Pr</i>	—	1
	<i>th st e Pr(Cy)</i>	22	42		<i>(Cy)</i>	—	2
4	<i>ru h th st V5</i>	—	7	4.6	<i>e(Cy)</i>	—	1
	<i>e Pr(Cy)</i>	1	1	5.6	<i>ru h th st Pr(Cy)</i>	3	11
5	<i>ru h th st(Cy)</i>	38	71		<i>V5 e</i>	4	9
	<i>V5 e Pr</i>	44	81	1.2.5	<i>ru th st(Cy)</i>	—	5
6	<i>ru h th st e(Cy)</i>	51	61		<i>h V5 e Pr</i>	1	5
	<i>V5 Pr</i>	66	99	1.2.6	<i>ru th st e(Cy)</i>	3	4
1.2	<i>ru th st e Pr(Cy)</i>	4	11		<i>h V5 Pr</i>	—	3
	<i>h V5</i>	1	7	1.5.6	<i>ru V5 e</i>	1	8
1.4	<i>h th st V5</i>	—	1		<i>h th st Pr(Cy)</i>	—	2
1.5	<i>ru V5 e Pr</i>	14	31	2.4.5	<i>ru h(Cy)</i>	—	1
	<i>h th st(Cy)</i>	7	25	2.5.6	<i>ru h V5 e</i>	—	3
1.6	<i>ru V5 Pr</i>	22	38		Total	712	1383
	<i>h th st e(Cy)</i>	7	31				
2.3	<i>ru h st e Pr(Cy)</i>	—	1				
2.5	<i>ru h V5 e Pr</i>	8	26				
	<i>th st(Cy)</i>	9	17				

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test (1)	21.0	11.2	0.3	0.3	18.3	23.8
(2)	28.0	15.8	0.1	1.0	21.5	22.3
Control	31.0	24.1	0.5		29.8	23.1
Standard	26.5	15.7	1.8		26.7	19.3

From this table two things may be observed. First, as in the preceding cases, the restriction of crossing-over to the chromosome under consideration allows a more nearly normal amount of crossing-over in the latter, excepting in this case in the *e-Pr* region. Under these circumstances, it is seen that, in this case, in which the break is very near the location of the spindle-fibre attachment, crossing-over is comparatively

little affected by the presence of the break and attached piece. Only, as may be observed in Table XIV, to follow, in the *st-cu* region itself is there any marked reduction for normal values. Elsewhere values are approximately normal. On the other hand, where crossing-over is not restricted to chromosome III, the presence of the competing synaptic elements results in a considerably more extended diminution of crossing-over.

A further detailed test to determine the location of the break in III in relation to *st* and *cu* was made by crossing *V5/Cy* females to *rucuca* males, and back-crossing the F_1 heterozygous females to *rucuca* males. The F_2 results for the loci *st*, *cu*, *e*, and *V5* are given in Table XIV.

TABLE XIV.

$$P_1: \frac{V5}{Cy} \text{ ♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{ ♂♂};$$

$$F_1 \text{ back-cross: } \frac{V5}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{ ♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{ru\ h\ th\ st\ cu\ sr\ e^s\ ca} \text{ ♂♂}.$$

	<i>st</i>	<i>cu</i>	<i>e_s</i>
	1	2	3
	<i>V5</i>		

(Only *st*, *cu*, *e^s*, and *V5* observed.)

0	<i>st cu e^s</i>	324	1.2	<i>st V5 cu e^s</i>	—
	<i>V5</i>	535		+	1
1	<i>st V5</i>	7	1.3	<i>st V5 e^s</i>	1
	<i>cu e^s</i>	4		<i>cu</i>	—
2	<i>st</i>	4	2.3	<i>st e^s</i>	1
	<i>V5 cu e^s</i>	6		<i>V5 cu</i>	2
3	<i>st cu</i>	81		Total	1065
	<i>V5 e^s</i>	99			

Percentages of crossing-over.

Region	1	2	3
Test	1.2	1.2	17.2
Standard		6.0	20.7

The above data locate the break in III of *V5* at a point about 3 units to the right of *st*, or approximately at locus 47.0. As in the case of *V4*, this is probably to the left of the spindle fibre, and leads us to believe that the connections of the pieces produced by the breaks are again II *L* to III *L* and II *R* to III *R*, as is confirmed below.

Connections of pieces in V5.

Males which were *V5 st Pr/S st* were crossed to females which were *V5/st*. The results found in F_1 are presented in Table XV.

The classes resemble in their respective frequencies those of Tarnished and Rosy; and the connections are therefore evidently also the same,

being namely, II *L* to III *L* and II *R* to III *R*. The excessive number of individuals in the *V5/Pr* class is again due to the inclusion of *V5/V5 Pr* homozygotes. The further test, now in progress, in which *V5 st Pr/Cy* males are being crossed to *V5 st/st* females, will throw further light on the existence of the extremely aneuploid gametes.

TABLE XV.

P_1 : *V5/st* ♀♀ × *S V5 Pr st/st* ♂♂.

(See Text-fig. 5.)

<i>V5 st Pr</i>	381	Orthoploid
<i>S V5</i>	318	
<i>S st</i>	370	
<i>S V5 Pr</i>	123	Moderately aneuploid
<i>V5 st</i>	187	
<i>V5 Pr</i>	32	Others (including <i>V5/V5 Pr</i> ,
<i>S V5 st</i>	4	cross-overs, and extremely
		aneuploid individuals)
Total	1415	

TESTS INVOLVING *V6*.

V6/Cy females were crossed to *S/Cy D/C_{IIIx}* males, and the F_1 males which were *V6/Cy D* were crossed to normal females. In the F_2 generation there was no recombination between *V6* and *D*, showing that a translocation between II and III was present.

This variegated mosaic mutant came originally from a stock of brown X-rayed by Moore, and therefore contained *bw* in chromosome II. The test with the stock of Bridges' Pale translocation showed it was in the region covered by the piece carried as excess. It is therefore to the right of arc, and since it closely resembles the other allelomorphs of brown, is in all likelihood a member of the series. But before final tests could be made, the stock reverted to a normal eye colour, rendering further work with it impossible.

Crossing-over in the second chromosome.

V6/Cy females were crossed to *Brisple/Cy* males, and the F_1 heterozygous females (*V6/Brisple*) were back-crossed to *apl/Cy* males. The F_2 results are given in the following table.

These data show a very remarkable condition, in which there is very little crossing-over in either arm of II. The double cross-overs and the single cross-overs are about equally numerous. This eliminates inversions as a means of explanation; nor can any other explanation be advanced at present.

98 *Mosaic Eye-Colour Mutants in Drosophila melanogaster**Crossing-over in the third chromosome.*

V6/Cy females were crossed to *Prple/Mo1* males, and the F_1 *V6/Prple* females were back-crossed to *rucuca/DC_x* males. The results in the F_2 generation are given in Table XVII.

In *V6*, as seen from that table, there is practically no crossing-over in the left arm of III. This condition therefore resembles III of Tarnished; and it may be surmised that in the present case too there is a

TABLE XVI.

$$P_1: \frac{V6}{Cy} \text{♀♀} \times \frac{al\ dp\ Bl\ c\ px\ sp}{Cy} \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V6}{al\ dp\ Bl\ c\ px\ sp} \text{♀♀} \times \frac{al\ dp\ b\ pr\ c\ px\ sp}{Cy} \text{♂♂}.$$

	<i>al</i>	<i>dp</i>	<i>Bl</i>	<i>c</i>	<i>px</i>	<i>sp</i>	
	1	2	3	4	5	6	
	<i>V6</i>						
	(Cy flies discarded.)						
0	<i>al dp Bl c px sp</i>	387			2.3	<i>al dp c px sp</i>	11
	<i>V6</i>	384				<i>Bl V6</i>	6
1	<i>al V6</i>	3			2.4	<i>al dp px sp</i>	4
	<i>dp Bl c px sp</i>	3				<i>Bl c V6</i>	3
2	<i>al dp V6</i>	10			3.4	<i>al dp Bl px sp</i>	7
	<i>Bl c px sp</i>	10				<i>c V6</i>	8
3	<i>al dp Bl V6</i>	7			3.5	<i>c px V6</i>	1
	<i>c px sp</i>	14			1.2.3	<i>dp c px sp</i>	4
4	<i>al dp Bl c V6</i>	1			1.2.4	<i>al Bl c V6</i>	2
	<i>px sp</i>	9					
1.2	<i>al Bl c px sp</i>	2				Total	876

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test	1.6	6.0	6.6	3.9	0.1	0.0
Standard	13.0	41.7	20.8	25.0	4.0	2.5

translocation of this arm, with the break some distance to the left of the spindle fibre.

V6 mutated frequently to normal, so that the stock had to be constantly purified. It is interesting to note that, if this mutant is an allelomorph of brown, it was produced by X-rays from the brown gene itself; and reverts to normal. During the summer of 1931 the variegated condition and the brown coloration were unfortunately entirely lost. But the reverted stock was examined and found to maintain itself in balance with *Cy*, and to give no recombinations with *Cy D*. It was therefore crossed to *Prple/Mo1*, and the heterozygous female offspring back-crossed exactly as before. The result was unexpected. It was found that there

was now no crossing-over at all in III, excepting for seven cases between *st* and *e*, one between *e* and *Pr*, and one double cross-over individual involving both these regions, in a total of 1008 individuals. Further tests are being made.

TESTS INVOLVING GRAPE (*Gr*).

Gr/Cy females were crossed to *S/Cy D/C_{IIIx}* males, and the F_1 *Gr/Cy D* males were then crossed to normal females. Grape showed no recombination with either *Cy* or *D*, showing that a translocation between II and III

TABLE XVII.

$$P_1: \frac{V6}{Cy} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ e\ Pr}{MoI} \text{ } \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{V6}{ru\ h\ th\ st\ e\ Pr} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{DC_x} \text{ } \text{♂♂}.$$

<i>ru</i>	<i>h</i>	<i>th</i>	<i>st</i>	<i>e</i>	<i>Pr</i>
1	2	3	4	5	6
V6					

(Dichaete flies disregarded.)

0	<i>ru h th st e Pr</i>	185	6	<i>ru h th st e</i>	77
	<i>V6</i>	146		<i>V6 Pr</i>	145
1	<i>ru V6</i>	1	2.4	<i>th st V6</i>	2
2	<i>th st e Pr</i>	1	2.5	<i>ru h V6 e Pr</i>	2
3	<i>ru h th V6</i>	1	3.6	<i>st e</i>	1
	<i>st e Pr</i>	1	4.5	+	2
4	<i>ru h th st V6</i>	2	5.6	<i>ru h th st Pr</i>	31
	<i>e Pr</i>	4		<i>V6 e</i>	11
5	<i>ru h th st</i>	75	4.5.6	<i>Pr</i>	1
	<i>V6 e Pr</i>	105			
Total					793

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test	0.3	0.6	0.4	1.4	28.7	33.6
Standard	26.5	15.7	1.8	26.7	19.3	
Control	31.0	24.1	0.5	29.8	23.1	

was present, and that Grape was located in either II or III. As will be recalled (Glass, 1933), it has been shown to be an allelomorph of the gene pink, in the third chromosome.

Crossing-over in the second chromosome.

Gr/Cy females were crossed to *Brisple/Cy DC_x* males, and the female offspring which were *Gr/Brisple DC_x* were back-crossed to *apl/Cy* males. The F_2 results are given in Table XVIII.

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Again we find the exceptionally high crossing-over values for the *b-Bl* region characteristic of the Brisple stock, and the relatively lower values for the *dp-b* region. As in the case of the brown allelomorphs pre-

TABLE XVIII.

$P_1:$	$\frac{Gr}{Cy}$	$\text{♀♀} \times$	$\frac{al\ dp\ b\ Bl\ c\ px\ sp}{Cy}$	$\frac{DC_x}{\text{♂♂}};$	
F_1 back-cross:	$\frac{Gr}{al\ dp\ b\ Bl\ c\ px\ sp\ DC_x}$	$\text{♀♀} \times$	$\frac{al\ dp\ b\ pr\ c\ px\ sp}{Cy}$	$\text{♂♂}.$	
	$\frac{al\ dp\ b\ Bl\ c\ px\ sp}{1\ 2\ 3\ 4\ 5\ 6\ 7}$				
	Gr				
	(Curly flies disregarded.)				
0	$al\ dp\ b\ Bl\ c\ px\ sp\ D$	72	2.5	$al\ dp\ px\ sp\ D$	9
	Gr	366		$b\ Bl\ c\ Gr$	4
1	$al\ Gr$	62	3.4	$al\ dp\ b\ c\ px\ sp\ D$	10
	$dp\ b\ Bl\ c\ px\ sp\ D$	9		$Bl\ Gr$	23
2	$al\ dp\ Gr$	172	3.5	$Bl\ c\ Gr$	3
	$b\ Bl\ c\ px\ sp\ D$	148	4.5	$c\ Gr$	1
3	$al\ dp\ b\ Gr$	52	1.2.4	$al\ b\ Bl\ Gr$	7
	$Bl\ c\ px\ sp\ D$	72		$dp\ c\ px\ sp\ D$	5
4	$al\ dp\ b\ Bl\ Gr$	52	1.3.4	$al\ Bl\ Gr$	2
	$c\ px\ sp\ D$	172		$dp\ b\ c\ px\ sp\ D$	3
5	$al\ dp\ b\ Bl\ c\ Gr$	4	1.3.5	$dp\ b\ px\ sp\ D$	2
	$px\ sp\ D$	25	2.3.4	$al\ dp\ Bl\ Gr$	4
1.2	$al\ b\ Bl\ c\ px\ sp\ D$	12		$b\ c\ px\ sp\ D$	5
	$dp\ Gr$	27	2.3.5	$al\ dp\ Bl\ c\ Gr$	1
1.3	$al\ Bl\ c\ px\ sp\ D$	16		$b\ px\ sp\ D$	2
	$dp\ b\ Gr$	21	3.4.5	$Bl\ px\ sp\ D$	1
1.4	$al\ c\ px\ sp\ D$	19	1.2.3.4	$al\ b\ c\ px\ sp\ D$	2
	$dp\ b\ Bl\ Gr$	14		$dp\ Bl\ Gr$	1
1.5	$al\ px\ sp\ D$	3	2.3.4.5	$b\ c\ Gr$	1
2.3	$al\ dp\ Bl\ c\ px\ sp\ D$	7			
	$b\ Gr$	29		Total	1526
2.4	$al\ dp\ c\ px\ sp\ D$	35			
	$b\ Bl\ Gr$	51			

Percentages of crossing-over.

Region	1	2	3	4	5	6	7
Test	13.4	34.2	16.2	26.7	3.7	0.0	0.0
Standard	13.0	35.5	6.2	20.8	25.0	4.0	2.5

viously discussed, the effect of the break upon crossing-over extends into the *c-px* region.

Crossing-over in the third chromosome.

Gr/Cy females were next crossed to *Cy Prple/Mo1* males, and the *F*₁ females which were *Gr/Cy Prple* were back-crossed to *rucuca/DC_x* males. The results obtained in *F*₂ are given in Table XIX.

Crossing-over values for the third chromosome of Grape are all normal or very similar to those in the control. This is further evidence to show

TABLE XIX.

$$P_1: \frac{Gr}{Cy} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ e\ Pr}{MoI} \text{ } \text{♂♂};$$

$$F_1 \text{ back-cross: } \frac{Gr}{Cy} \text{ } \text{♀♀} \times \frac{ru\ h\ th\ st\ cu\ sr\ e^s\ ca}{DC_x} \text{ } \text{♂♂}.$$

<i>ru</i>	<i>h</i>	<i>th</i>	<i>st</i>	<i>e</i>	<i>Pr</i>
1	2	3	4	5	6
<i>Gr</i>					

(Dichaete flies disregarded.)

0	<i>ru h th st e Pr Cy</i>	151	4.5	<i>ru h th st Gr e Pr</i>	6
	<i>Gr</i>	374		<i>Cy</i>	3
1	<i>ru Gr</i>	111	4.6	<i>ru h th st Gr Pr</i>	6
	<i>h th st e Pr Cy</i>	94	5.6	<i>ru h th st Pr Cy</i>	15
2	<i>ru h Gr</i>	106		<i>Gr e</i>	35
	<i>th st e Pr Cy</i>	126	1.2.4	<i>ru th st Gr</i>	2
3	<i>ru h th Gr</i>	3	1.2.5	<i>ru th st Cy</i>	8
	<i>st e Pr Cy</i>	3		<i>h Gr e Pr</i>	13
4	<i>ru h th st Gr</i>	11	1.2.6	<i>ru th st e Cy</i>	7
	<i>e Pr Cy</i>	15		<i>h Gr Pr</i>	11
5	<i>ru h th st Cy</i>	86	1.3.6	<i>h th Gr Pr</i>	1
	<i>Gr e Pr</i>	165	1.4.5	<i>ru Cy</i>	3
6	<i>ru h th st e Cy</i>	61		<i>h th st Gr e Pr</i>	3
	<i>Gr Pr</i>	152	1.4.6	<i>ru e Cy</i>	1
1.2	<i>ru th st e Pr Cy</i>	20		<i>h th st Gr Pr</i>	5
	<i>h Gr</i>	34	1.5.6	<i>ru Gr e</i>	7
1.3	<i>h th Gr</i>	1		<i>h th st Pr Cy</i>	10
1.4	<i>ru e Pr Cy</i>	7	2.3.6	<i>th Gr Pr</i>	1
	<i>h th st Gr</i>	5	2.4.5	<i>ru h Cy</i>	1
1.5	<i>ru Gr e Pr</i>	51		<i>th st Gr e Pr</i>	4
	<i>h th st Cy</i>	59	2.4.6	<i>th st Gr Pr</i>	1
1.6	<i>ru Gr Pr</i>	49	2.5.6	<i>ru h Gr e</i>	10
	<i>h th st e Cy</i>	52		<i>th st Pr Cy</i>	12
2.4	<i>ru h e Pr Cy</i>	1	4.5.6	<i>Pr Cy</i>	1
	<i>th st Gr</i>	7	1.2.5.6	<i>ru th st Pr Cy</i>	2
2.5	<i>ru h Gr e Pr</i>	41		<i>h Gr e</i>	3
	<i>th st Cy</i>	70	1.4.5.6	<i>h th st Gr e</i>	2
2.6	<i>ru h Gr Pr</i>	30			
	<i>th st e Cy</i>	55			
3.5	<i>st Cy</i>	1		Total	2114

Percentages of crossing-over.

Region	1	2	3	4	5	6
Test	26.5	26.7	0.5	4.0	28.8	25.0
Control	31.0	23.1	0.5		29.8	23.1
Standard	26.5	15.7	1.8		26.7	19.3

that when a break occurs close to the spindle fibre, the effect upon crossing-over of the break itself and the reattachment are very slight. This is

evident when, as in the present test, the reduction of crossing-over due to competing synaptic elements is eliminated by the presence of inversions in the chromosome not under consideration. It may also be noted that *Cy* always segregated from Grape; that is, there is no crossing-over between the point of the break, as marked by *Cy*, and Grape itself. This complete linkage of the break and the dominant mutant is further evidence, and from a different locus than that of brown, that they are in some way intimately related.

In order to study more closely the exact point of the break in III, *Gr/Cy* males were crossed to *rucuca* females, and the F_1 heterozygous females (*Gr/rucuca*) were back-crossed to *rucuca* males. The results for the loci *st*, *Gr*, *cu*, and *e* are given in Table XX.

TABLE XX.

$$P_1: \frac{Gr}{Cy} \delta \delta \times \frac{ru h th st cu sr e^s ca}{ru h th st cu sr e^s ca} \varphi \varphi;$$

$$F_1 \text{ back-cross: } \frac{Gr}{ru h th st cu sr e^s ca} \varphi \varphi \times \frac{ru h th st cu sr e^s ca}{ru h th st cu sr e^s ca} \delta \delta.$$

		$\frac{st}{1}$	$\frac{cu}{2}$	$\frac{e^s}{3}$		
		$\frac{Gr}{1 \quad 2 \quad 3}$				
0	$st cu e^s$	355			1.3	$st Gr e^s$
	Gr	475				cu
1	$st Gr$	8			2.3	$st e^s$
	$cu e^s$	8				$Gr cu$
2	st	15				
	$Gr cu e^s$	13				
3	$st cu$	106				
	$Gr e^s$	133				
					Total	1123

Percentages of crossing-over.

Region	1	2	3
Test	2.1	2.8	22.1
Standard		6.0	20.7

These data would locate Grape at a point about 2.6 units to the right of *st*, or at locus 46.6, to the left of the spindle-fibre attachment. But from the preceding table (XIX) a considerably higher value for the *st-Gr* region was found, namely, 4.0 units. This difference is undoubtedly partially due to the presence of cross-over suppressors for II in the previous test, which were absent here. The same effect is seen in the *cu-e* region. From the next section we see that the actual connections of the broken pieces in Grape are II *L* to III *R* and II *R* to III *L*, so that the break is actually to the right of the spindle fibre; this fact agrees with

the fact that the mutated locus, that of pink, is known to be in this position.

Connections of the broken pieces.

Males which were *st Gr Pr/S st* were crossed to females which were *Gr/st*. The results are given in Table XXI.

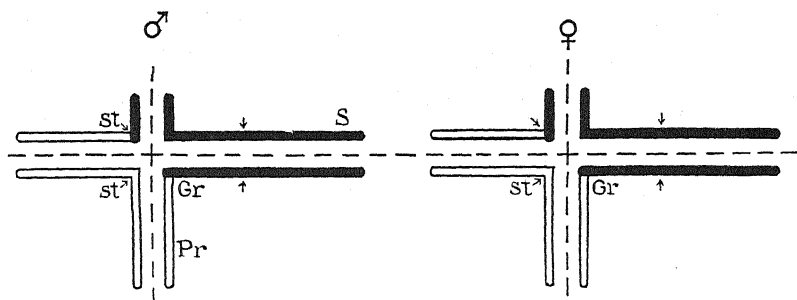
TABLE XXI.

P_1 : *Gr/st* ♀♀ × *S Gr Pr st/st* ♂♂.

(See Text-fig. 6.)

<i>st Gr Pr</i>	844	Orthoploid
<i>S Gr</i>	791	
<i>S st</i>	733	
<i>Gr Pr</i>	388	Moderately aneuploid
<i>S st Gr</i>	324	
<i>S Gr Pr</i>	4	Others (including extremely
<i>st Gr</i>	1	aneuploid individuals and
<i>S st Gr Pr</i>	10	others as described in text)
<i>Gr</i>	2	
Total	3097	

The fact that the moderately aneuploid classes are, in the case of Grape, *Gr Pr* and *S st Gr*, proves that the connections are as given in Text-fig. 6. The *S st Gr Pr* flies were tested by crossing them to *st* males



Text-fig. 6.

or females, and it was found that they were not really Star, but that a new eye mutation had arisen in the stock of Grape; it was a recessive resembling Star, and was responsible for the appearance of these individuals. Two of the *S Gr Pr* flies, when similarly tested, proved to be genuinely Star. The *Gr* individuals were also tested, and were found to be heterozygous for *st*, and so must have come from the union of two gametes each formed by the segregation of three chromosomes to one pole and one to the other, a three-chromosome gamete containing *Gr*

coming from the mother, and a one-chromosome gamete containing *st* coming from the father. Crossing-over in the female parent would have produced individuals indistinguishable from the moderately aneuploid individuals; this is different from the cases of all the allelomorphs of brown, in which the connections were different, and the cross-over individuals resembled the extremely aneuploid classes. Further tests with Grape, similar to those being carried out with Tarnished, *V4*, and *V5*, are being made.

TESTS INVOLVING *MOIRÉ 1 (Mo1)*.

Muller (1930*b*) remarks concerning *Mo1*, "it is located in the (homologue of the) left arm of the third chromosome, *in a region which has undergone an inversion*, since cross-overs between the left arm of a normal third chromosome and the homologous region bearing Moiré fail to occur, or, at any rate, to be visible." The present writer has done little further work upon *Mo1* at this time. He is informed, however, by Prof. Mohr, that Moiré is included within the region covered by Vein deficiency, that is, within the region of the third chromosome extending from about locus 10.6 to locus 23.0 on the standard map, a distance of 12.4 units. This evidence is based on the fact that the Vein/Moiré compound is lethal (Mohr, 1933).

Mo1 was later treated with X-rays again, by Muller, in order to provide a non-cross-over stock for III containing a dominant marker, and a translocation between II and III was produced. This stock, *Mo1_x*, undergoes hardly any crossing-over in II, except in the left arm; and hardly any in III, except in the region of claret. It has been used extensively for balancing stocks of third chromosome characters for that reason. But since this new translocation had nothing to do with the character *Mo1* itself, which was already present, it is not further considered here.

TESTS INVOLVING *MOIRÉ 2 (Mo2)*.

It has already been shown that the *Mo1/Mo2* compound is inviable (Glass, 1933), a proof that they are allelomorphs. *Mo2* may therefore be considered as lying also in the left arm of III. *Mo2/Cy* females were crossed to *S/Cy D/C_{IIIx}* males, and the *Mo2/Cy D* male offspring were then crossed to normal females. In *F*₂ there was no recombination of *Mo2* with either *Cy* or *D*, showing that a translocation between II and III was present.

mately at the locus of *Bl*), and a mutual translocation which involves the connections II *L* to III *R* and II *R* to III *L*. Placing the break to the left of the spindle fibre would help to account for the lowered crossing-over between *dp* and *Bl*, and the normal crossing-over throughout the right arm of II.

It is unfortunately impossible to test this hypothesis completely, since the situation in III has been so changed that practically no crossing-over occurs in it. Yet what evidence has been obtained is in accordance with the view just set forth. When *Mo2/Cy* females were crossed to *Prple/Mo1* males, and the F_1 *Mo2/Prple* females were back-crossed to *rucuca/DC_w* males, there were only eight cross-overs in a total of 655 individuals. These were:

<i>ru h th st</i>	2
<i>Mo2 e Pr</i>	2
<i>ru h th st Pr</i>	2
<i>Mo2 e</i>	1
<i>Mo2 h</i>	1

All these are double cross-overs, except possibly the *Mo2 e Pr* and the *ru h th st*; and even these may be double cross-overs, if the second point of breakage were to the right of *Pr*. There is evidently an inversion involving the left arm also, since only one cross-over was found in it, and it is a double one involving the hairy locus only. The appearance of this individual indicates that the locus of *Mo* is not very close to hairy, and since we have seen above that it is to the left of hairy, it is presumably nearer locus 10 than locus 20.

Mo2 is similar to *V6* in that it reverts frequently to normal. With some difficulty, in this case the original stock, as well as the reverted type, has been preserved. When crossing-over in III in the reverted stock was tested, it was found to be indistinguishable from that in the variegated *Mo2*. The second chromosome of the reverted stock has not yet been tested.

GENERAL DISCUSSION.

From the preceding data several conclusions have been drawn. It will be well to gather these into a few concise statements. We have so far found three loci which are subject to dominant mosaic eye-colour mutations. The mosaicism is not alike, however, at these three loci, but is of a characteristic type for each locus. At the loci of *bw* and *Mo* eight different characters have been studied, of which three were found to be associated with inversions only, and five with mutual translocations.

But from crossing-over data, we find that of the inversions one case (Plum) involves two simultaneously; while among the mutual translocations we find one case (Tarnished) in which there is a secondary translocation, one case (*Mo2*) in which there are two inversions besides the translocation, and a third case (*V6*) which is doubtful, but possibly contains one or even two inversions. This means that we have in reality about seven inversions to five cases of mutual translocations. When we add the case of Grape, also a mutual translocation, we obtain the ratio 7:6, or, considering the small number of cases, 1:1. If, when two breaks occur simultaneously, it is a matter of chance whether they occur in one chromosome or in two, we should expect to obtain equal numbers of inversions and mutual translocations. This seems to be the case.

Tests for both second and third chromosomes of the mutual translocations *V4* and *V5* show that crossing-over is markedly increased in every case in the one chromosome when it is simultaneously inhibited in the other. This fully confirms the findings of Dobzhansky and Sturtevant (1931). The crossing-over tests show further that where the break is in one of the regions close to the spindle fibre, crossing-over is not materially affected in either arm of the chromosome (see II of *Mo2*, and III of *V3*, *V4*, *V5*, and *Gr*). But when the break occurs at some distance from the spindle fibre, and even as close as 2 units from the end of the chromosome, the effect of the break is large, and its effect at any one point apparently decreases with increasing distance from the break. This confirms the findings of Dobzhansky (1932) on translocations from II to the Y-chromosome. We may then consider the effects of breaks and re-attachments upon crossing-over as being of a triple nature. First, there is the diminution of crossing-over which is produced by contrary attractions on the two parts of a compound chromosome (see Dobzhansky, 1931*a*). This may be eliminated by inserting into the test a non-crossing-over chromosome which exerts no attraction on its homologous parts, and leaves the other portion of the compound chromosome free to synapse. Secondly, there is the effect of the attached portion of chromatin, which may exert a mechanical hindrance of some sort to crossing-over, even though it is not being attracted in synapsis itself. Thirdly, there is the effect of the break itself. When the first factor reducing crossing-over is eliminated, the other two are studied in conjunction. If now the translocation can be studied in the homozygous form, these factors may be separated also.

All of the tests for crossing-over in III among the brown allelomorphs *V3*, *V4*, *V5*, and *V6* show that it is broken close to the spindle fibre (in

terms of the genetic map), and in the three cases which could be tested, it was in each case broken to the left. In Grape, again, the break is very close to the spindle fibre in III. Of the translocations described by Dobzhansky and Sturtevant (1931), there are five breaks in the middle of the second and third chromosomes in the four cases. Of the cases reported by Van Atta (1932) which are translocations, Salmon is broken in the middle of both II and III. Other data are in accordance (unpublished data of Muller), and seem to indicate that more than half of the autosomal breaks occur in the middle of the chromosomes, that is, at a point genetically close to the spindle-fibre attachment. Cytologically, as Dobzhansky (1930*b*) has shown, we have reason to believe that the regions just to either side of the spindle-fibre attachments in II and III are much longer than their genetic distance would indicate. But even so, these regions together do not amount to more than one-fifth of the total length of the chromosome, and yet they contain over half of the breaks. This must be taken to mean that they are really regions of weakness, especially liable to breakage. The fact that so many breakages have occurred just at brown (although we must remember that these are specially selected), including the six cases discussed here and the six "Dilutes" of Van Atta, and also Grape, which is broken at very near the same point, may indicate the existence of another weak point.

All of these variegated dominant mutants are, as far as may be determined, in some way related to the presence of a break at the locus concerned. Yet the reversions of *V6* and *Mo2* to normal show that the variegation is not determined solely by the translocation or inversion present; since in these two cases translocations remained after the eye colour had reverted to normal.

The relationship of the sizes of the respective pieces translocated, to the ratios of the orthoploid and aneuploid classes of individuals and gametes is an interesting question. Dobzhansky and Sturtevant (1931) found that when both chromosomes were broken in the middle, the aneuploid classes were formed in equal numbers, but there were many more orthoploid individuals than aneuploids. Owing to viability complications, it is impossible to determine the ratio from their data with any validity. They found, too, that when one chromosome was broken in the middle and the other at a point remote from the middle, although orthoploids still exceeded aneuploids, the aneuploid classes themselves differed, the ones formed by the separation of the greater masses of homologous chromatin being numerous, the ones in which the greater masses of homologous chromatin underwent non-disjunction failing to appear.

The present cases are all of this latter type, and from them the ratio of orthoploid individuals to aneuploid individuals per class was found to be 2:1 (see *V3*, *V4*, *V5*, and *Gr*). The ratios of the gametes, orthoploid to aneuploid, for *V3*, *V4*, *V5*, and *Gr*, respectively, are 1.4:1, 1.4:1, 1.5:1, and 1.5:1. It is as yet uncertain whether or not the extremely aneuploid gametes are ever formed in these cases.

SUMMARY.

1. Six dominant mosaic eye-colour mutants of the brown locus (II-104.3) have been studied. Four of these six are associated with mutual translocations, two with inversions only. In each case one of the breaks is at or very near the locus concerned.

2. One dominant allelomorph (Grape) of the mutant pink (III-48.0) has been studied. It is associated with a mutual translocation, and one break is at or very close to the locus concerned.

3. Two dominant allelomorphic mosaic eye colours (Moiré) located in the left arm of III (between 10.6 and 21.1) are associated, one with an inversion, the other with a mutual translocation.

4. Flies have been obtained retaining the mutual translocation, but with normal eye colour, in two cases (*V6* and *Mo2*).

5. More than half of all chromosomal breaks occur in the immediate vicinity (genetic) of the spindle-fibre attachments in II and III.

6. When a break occurs in a sub-terminal region, crossing-over is affected in that arm in an amount which varies inversely with the distance from the break.

7. When a break occurs near the spindle fibre, crossing-over is very little affected in either arm.

8. Dobzhansky and Sturtevant's finding that crossing-over in a mutual translocation is increased in one chromosome when it is simultaneously inhibited in the other, is confirmed.

9. The ratio of orthoploid to aneuploid gametes in the cases studied is 1.4 (or 1.5):1. The existence of extremely aneuploid gametes is uncertain.

10. It is shown that in certain cases two chromosomes must have undergone synapsis in the form of a ring. This shows that crossing-over need not always begin near the spindle fibre and progress outwards, nor at the distal end and progress toward the spindle fibre.

11. A method is described for accurately determining the location of the spindle-fibre attachment with reference to known neighbouring genes by means of the segregation of gametes in a mutual translocation.

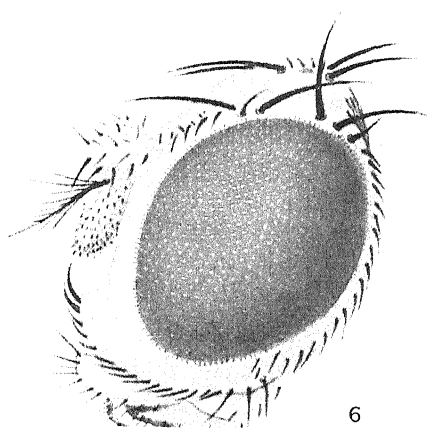
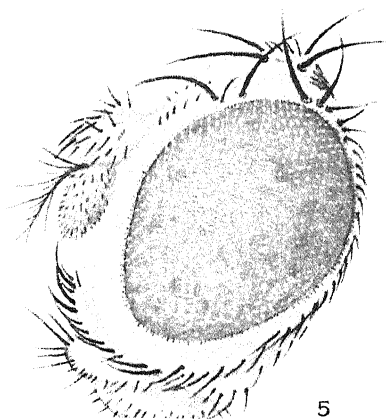
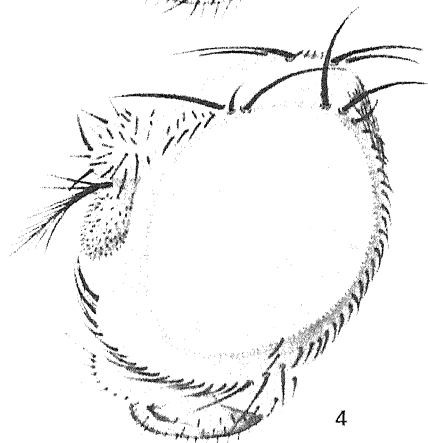
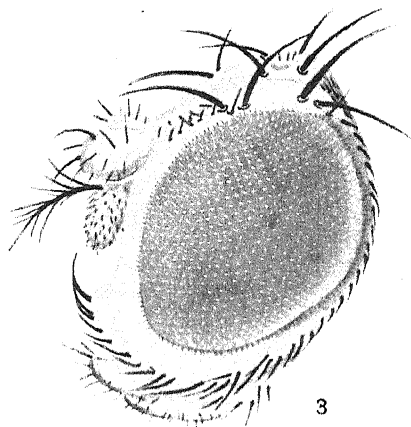
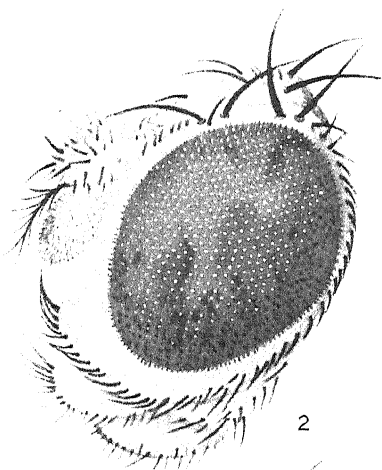
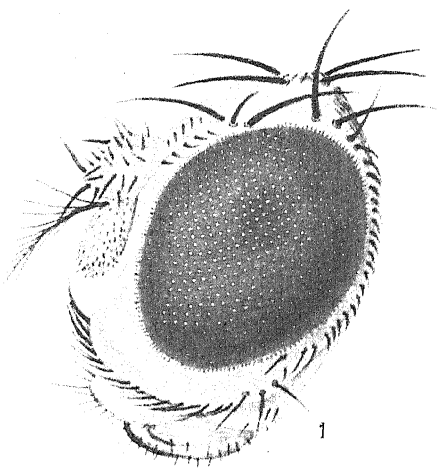
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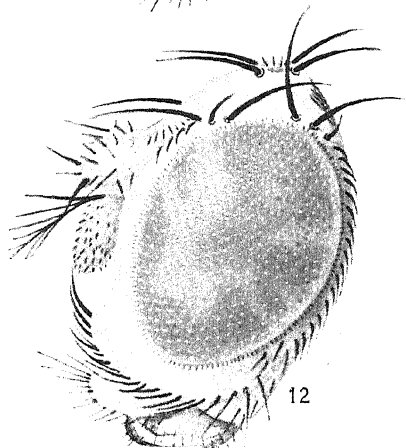
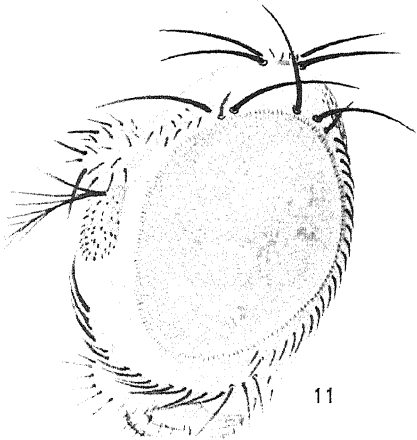
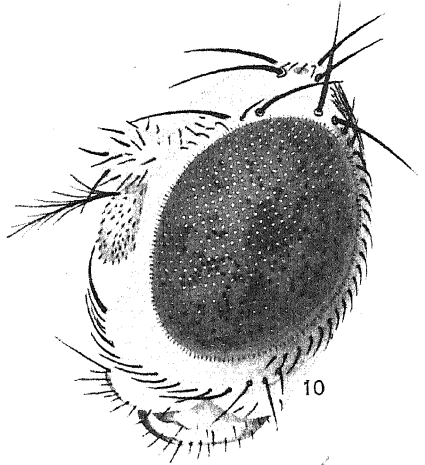
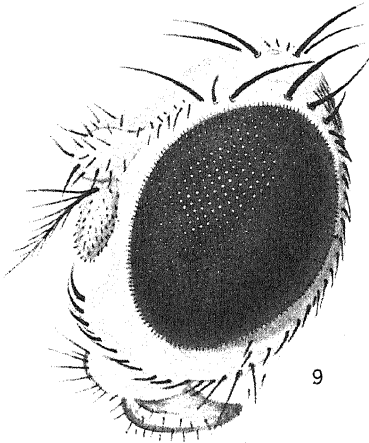
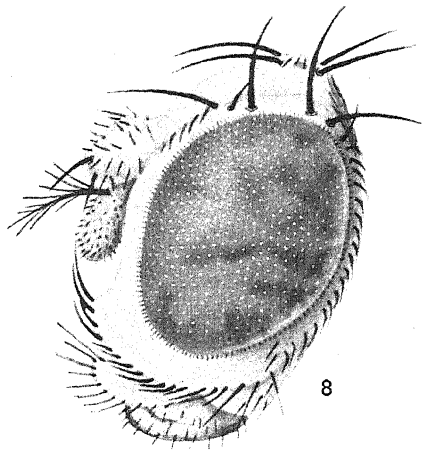
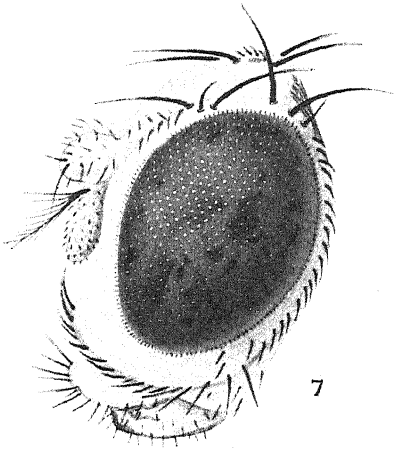
12. A means is described for testing Serebrowsky's hypothesis that translocations and inversions occur by exchanges at points of contact between intertwisting chromosomes or portions of chromosomes.

The author wishes to express his deep indebtedness for much assistance and advice in the work done on this problem to Dr H. J. Muller. The work has been made possible through a research fellowship granted by the Zoology Department of the University of Texas. The author is also indebted for help and advice during the revision of this work (which is constructed from a thesis presented in partial fulfilment of the requirements for Doctor of Philosophy at the University of Texas), to Prof. Otto L. Mohr, of the Anatomical Institute of the University at Oslo, Norway.

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EXPLANATION OF PLATES V—VI.

PLATE V.

- Fig. 1. Normal eye colour (+/+ and *st*/+). The single black area appears to be beneath the surface of the eye, and shifts its position with every change in the angle at which the light falls upon the eye.
- Fig. 2. Heterozygous Rosy (*V4*/+). An average individual, the number of the black spots, which appear to be superficial, varying from very few to many more than here depicted. The brown colour of the eye may vary from a rosy tint to a reddish brown. Individuals similar to this type may be found in cultures of Tarnished, *V5*, and sometimes even Plum. The extinct *V6* varied in appearance from this type to that in Figs. 7 and 10. Age about one week.
- Fig. 3. Homozygous Rosy (*V4*/*V4*). The spots are very faint, often invisible, and when apparent seem to lie deep in the eye. There is a translucency not found in the heterozygous types. This type is also characteristic of homozygous *V1*, *V2*, *V3*, and *V5*.
- Fig. 4. Homozygous Rosy with homozygous scarlet (*V4*/*V4 st/st*). The colour varies from a pure white to a cream tint. At first no spots are visible, but with increasing age a few very small and scattered yellow or orange spots become apparent. This type is characteristic also of the similar compounds *V3*/*V3 st/st* and *V5*/*V5 st/st*. *V1* has never been observed in this compound, but is probably also similar. *V2*/*V2 v/v* is pure white to cream, with no spots.
- Fig. 5. Heterozygous Rosy with homozygous scarlet (*V4*/+ *st/st*). The substitution of vermilion for scarlet gives similar results. This figure may be regarded as typical for such compounds of any of the dominant allelomorphs of brown, at the age of about one week after emergence of the imago. The spots vary in size from one to several ommatidia, and are distributed at random. A comparison with Fig. 2 shows that the

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presence of *st/st* changes the colour of the spots from black to orange, and the colour of the background from brown to yellow.

Fig. 6. Homozygous scarlet (*st/st*).

Fig. 7. Heterozygous Tarnished (*V3/+*). On the average, a darker brown colour than in *V4*, with more numerous black spots. But individuals similar to this figure may be found in cultures of *V4* and *V5*. Age about one week.

Fig. 8. Heterozygous Tarnished with homozygous scarlet (*V3/+ st/st*). Over ten days after emergence. This is also quite typical of the other dominant allelomorphs of brown in the corresponding compounds with scarlet, and at a corresponding age.

Fig. 9. Heterozygous Tarnished (*V3/+*). Over ten days old. The ground colour of the eye has become so dark that the spots appear very faint, or cannot be discerned at all. *V4* and *V5* are similar to this at a like age.

Fig. 10. Heterozygous *V5* (*V5/+*). On the average, a redder ground colour than in *V3* or *V4*; although individuals similar to this may be found in cultures of *V3* frequently, and in cultures of *V4* occasionally. *V6* was similar in appearance to this type.

Fig. 11. Heterozygous *V5* with homozygous scarlet (*V5/+ st/st*); less than three days after emergence. *V3* and *V4* in corresponding compound and at same age also look like this figure and the next.

Fig. 12. Same as Fig. 11; to show extreme variation in number of spots (and consequently, in colour) to be observed in a single culture.

PLATE VI.

Fig. 13. Heterozygous Plum (*V1/+*). Less than three days after emergence. The ground colour is very variable, occasionally appearing almost as purplish as Grape (see Fig. 21). The spots are relatively few, and are rather faint; while dim blackish areas can sometimes be observed deep in the eye.

Fig. 14. Heterozygous Plum (*V1/+*). Over ten days after emergence. Often has a more purplish tone than in the individual here figured.

Fig. 15. Heterozygous Plum with homozygous scarlet (*V1/+ st/st*). Under three days old.

Fig. 16. Heterozygous Discoloured (*V2/+*). Very similar to Plum, but not quite so purplish. Young.

Fig. 17. Heterozygous Discoloured with hemizygous (male) vermilion (*V2/+ v*). Young. The ground colour is a little lighter yellow than in the similar compounds with other dominant brown allelomorphs figured above; and the spots are browner and less orange.

Fig. 18. Moiré 1, heterozygous (*Moi/+*). Young. The black spots seem few and are only seen with some difficulty. Recognition is easiest by means of the shimmering iridescent areas which shift as the eye is moved in the light, and which appear to be below the surface of the eye. Moiré 2, now lost, was similar in appearance.

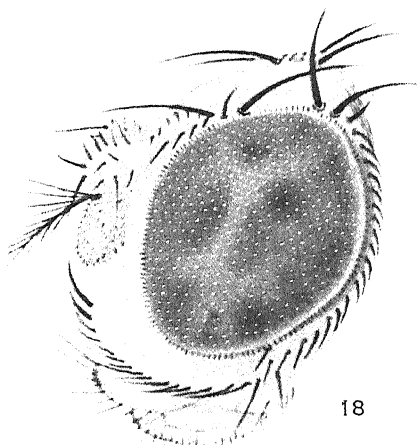
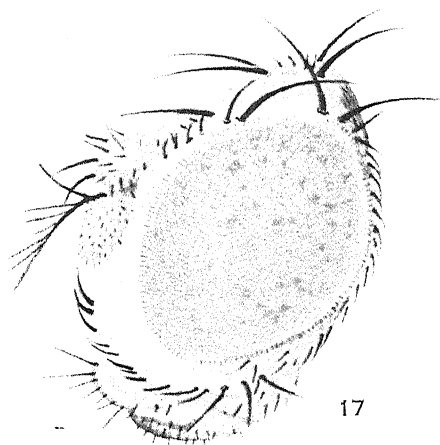
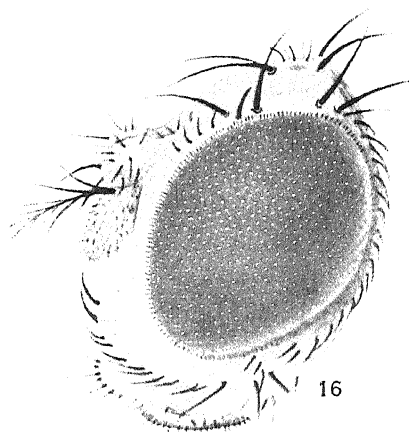
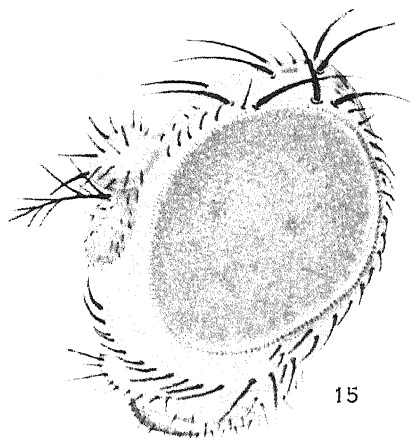
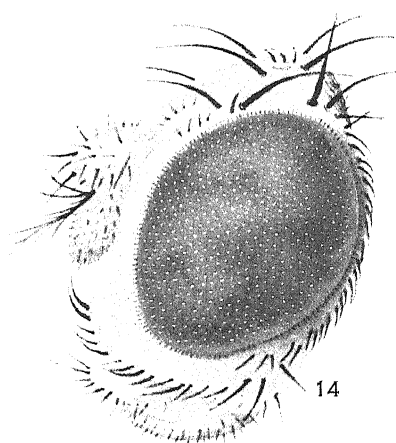
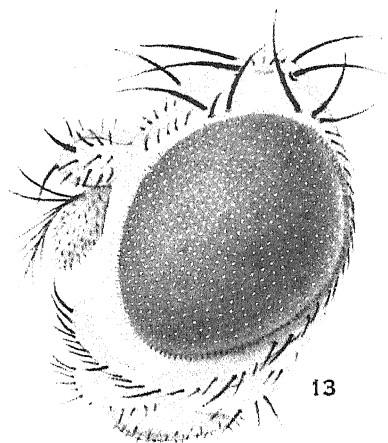
Fig. 19. Moiré 1, heterozygous, with hemizygous vermilion (*Moi/+ v*). Young. The eye shows the black spots most distinctly along the edge. The ground-colour is scarlet or vermilion, not orange as in the allelomorphs of brown. The iridescent areas are present.

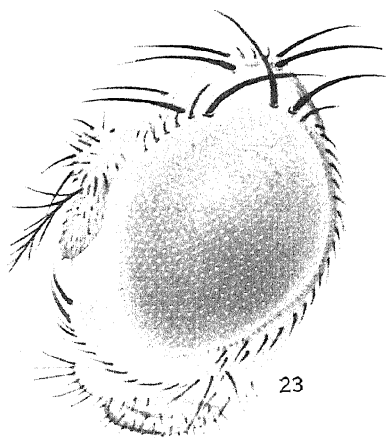
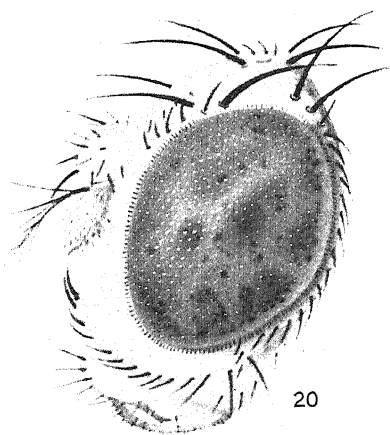
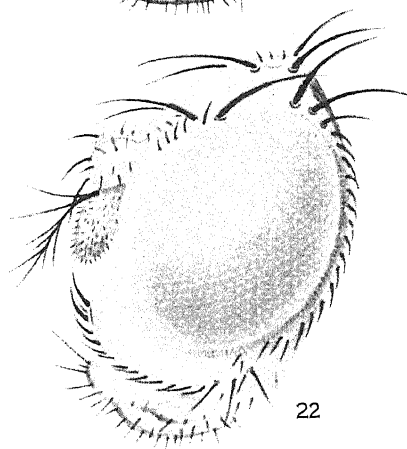
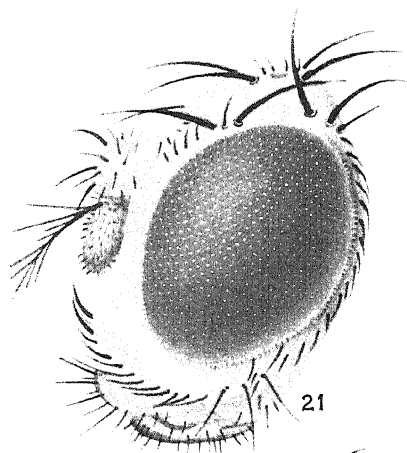
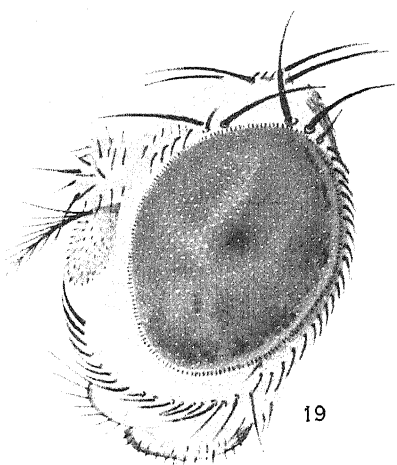
Fig. 20. Moiré 1, heterozygous (*Moi/+*). Old. The black spots appear very numerous and distinct, whereas in the allelomorphs of brown they disappear with age (see Fig. 9). The iridescent areas are still plain.

Fig. 21. Heterozygous Grape (*Gr/+*). A clear translucent colour, not deepening with age to any marked extent, and varying little from one individual to another.

Fig. 22. Heterozygous Grape with homozygous scarlet (*st Gr/st*). No spots are visible on the pale orange-yellow ground colour. But this itself varies in intensity over large areas. Compare Fig. 23.

Fig. 23. Same as Fig. 22, to show the extreme range of variation possible even between right and left eyes of the same individual.





THE CYTOLOGY OF CERTAIN INTERGENERIC HYBRIDS BETWEEN *FESTUCA* AND *LOLIUM*¹.

By F. H. PETO.

(Welsh Plant Breeding Station, Aberystwyth.)

(With Plate VII and Sixty-two Text-figures.)

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INTRODUCTION.

THE genera *Festuca* and *Lolium* differ strikingly in certain morphological characters and in consequence have been widely separated in many classifications. Nevertheless, certain species of these two genera can be hybridised. Garton (McAlpine, 1898) appears to have been the first worker to have done this, but very little information concerning the resulting hybrids is available. In 1921 successful breeding work with these genera was carried out by Mr T. J. Jenkin at the Welsh Plant Breeding Station. Since that time he has developed the work extensively and a considerable amount of information with regard to the breeding affinities of the two plant groups has been accumulated (Jenkin, 1924, 1925, 1930, 1931 a).

The present investigation is mainly a cytological study of certain plants very kindly placed at my disposal by Mr Jenkin. These plants represented the following crosses and their derivatives:

¹ The financial assistance provided by the award of two consecutive Fellowships by the National Research Council of Canada has made this study possible.

Lolium perenne L. ($n = 7$) \times *Festuca pratensis* Huds. ($n = 7$).

Festuca pratensis Huds. ($n = 7$) \times (*Lolium perenne* \times *L. perenne* var. *multiflorum*) ($n = 7$).

Lolium perenne L. ($n = 7$) \times *Festuca arundinacea* Schreb. ($n = 21$).

Festuca gigantea Vill. ($n = 21$) \times *Festuca arundinacea* Schreb. ($n = 21$ or 22).

Additional plants were studied which were definitely identifiable as the supposed natural hybrid *Festuca loliacea* (*F. pratensis* \times *L. perenne*), and others which were provisionally identified as such.

Three methods of study were employed:

(1) The somatic chromosome numbers of all the plants were counted in the root tips. These data provide an indispensable check to meiotic interpretations in hybrids in which one deals with a series of plants which often differ by only one or two chromosomes. Observations on chromosome structure and fragmentation were also made.

(2) The meiotic studies constitute the most important line of investigation, since it is at this stage that evidences of hybridity are most pronounced. The degree of pairing between the parental complements in the F_1 is a good criterion of their genetic relationships. Until recent years the usual method of measuring the degree of pairing was to record the proportion of univalents to bivalents and higher associations. Since the chiasmatype theory was initiated by Janssens (1924), Darlington (1930*b*) and others have used the chiasma frequency as a more accurate measurement of chromosome relationship. Darlington has also shown that the pairing of chromosomes at metaphase depends on the formation of chiasmata amongst the associated chromatids at prophase. It has been demonstrated in species where there is no extreme variation in the size of chromosome, that the chiasma frequency is proportional to the length of the chromosomes paired at this stage (*Vicia*, Maeda (1930) and *Fritillaria*, Darlington (1930*b*)). The value of deductions from chiasma frequencies rests on the assumption that pachytene pairing is the result of some attraction between genetically similar parts of chromosomes. Therefore if a lower chiasma frequency is observed in a hybrid than in either parent, it is interpreted as being the result of a lower degree of homology between the pairing complements in the hybrid than had existed in either parent. This method of analysing chromosome association has been used extensively in this investigation.

Irregularities in pairing naturally give rise to abnormal behaviour throughout meiosis, and these often result in degeneration at various

stages. These abnormalities, as well as the stage at which degeneration is first observable, have been recorded.

(3) The chief concern in the study of hybrids is whether or not they can produce viable gametes. Without actual breeding tests it is possible to get some indication of the viability of the pollen by examining it just prior to its exsertion from the anther. The percentage of apparently good pollen was determined at this stage for all the plants in this study.

LITERATURE REVIEW.

Naturally occurring plants, intermediate in morphological characters between species of *Festuca* and *Lolium*, have for many years been classified by taxonomists as intergeneric hybrids. For instance, Ascherson and Graebner (1902) described plants classified by them as hybrids of the following types: *F. pratensis* \times *L. perenne*, *F. pratensis* \times *L. multiflorum*, *F. arundinacea* \times *L. multiflorum*, *F. gigantea* \times *L. perenne*. Holmberg (1930) has more recently described a plant of natural origin, which he believed to be a hybrid between *F. pratensis* and *L. multiflorum*, and Nilsson (1930) artificially hybridised *L. multiflorum* and *F. gigantea*.

There has been no previous work published on the cytology of these intergeneric hybrids, although numerous authors have worked on the cytology of the parental species. Evans (1926), Stählin (1929), Katterman (1930) and Radeloff (1930) published data on the chromosome numbers, and their counts, with one exception (*F. arundinacea*), were identical with those determined for the parental species used in this investigation. Church (1929) includes three species of *Festuca* in his study of the meiotic phenomena in Gramineae. In *Festuca rubra* he observed irregular disjunction of bivalents in the heterotypic division and occasional stranding of chromosomes in the adjacent mother cells during diakinesis and cytomyxis. About 25 per cent. of the pollen was found to be imperfect. Levitsky and Kuzmina (1927) have determined the chromosome number of a large number of species and varieties of *Festuca*. They used this information to assist in their systematic and phylogenetic studies. Avdulow (1931) adopted similar methods in making a comprehensive survey of the systematics in the whole of the Gramineae. The somatic chromosomes of four species of *Lolium* have been carefully observed by Faworski (1927), who attempted to correlate chromosome characters such as length with certain phenotypic characters. His work in this regard appears to be of little importance, but his observations on the details of chromosome structure in *Lolium perenne* form an interesting comparison with those made in the present study.

METHODS.

The root tips were fixed in Karpechenko's solution and La Cour's fixative No. 2-bE. The former was found to be fairly satisfactory in combination with Heidenhain's iron-haematoxylin and the latter was used with both haematoxylin and gentian violet¹.

In the fixation of the pollen mother cell material, the proper stage for collecting was quickly ascertained by Belling's aceto-carmin method. The florets adjacent to those found to be at the correct stage were then placed in Carnoy's solution for 1 min. and then direct into either medium Flemming's or Karpechenko's solutions. The flowering glumes were not removed, and so it was usually necessary to take out the air with a vacuum pump. The usual technique was employed in passing this material through the grades of alcohol and xylol into paraffin. All the pollen mother cell material was sectioned and stained at John Innes Horticultural Institution. The sections were cut at 20 μ . Gentian violet was used exclusively for this material and the schedule given by La Cour (1931) was followed closely. The fixation with both medium Flemming's and Karpechenko's solutions varied slightly with the different species. The latter was usually slightly superior, since the cytoplasm cleared more readily and the chromosomes appeared to take up the stain better. On the other hand the outline of the chromosomes fixed with medium Flemming's was particularly sharp.

¹ For a time some difficulty was experienced in getting the chromatin to stain heavily enough so that proper differentiation could be effected. This was particularly so when La Cour's fixative was used in combination with gentian-violet stain. Since this difficulty was not experienced in other cytological laboratories a local explanation was sought. After much experimentation the tap water was suspected as being responsible. The official analysis of the Aberystwyth water supply was compared with that supplying Cambridge University and John Innes Horticultural Institution. The Aberystwyth water supply gave an acid reaction, with a pH of approximately 6.0 and contained very little total solids in solution. Both the other water supplies were alkaline with a pH of approximately 8.0 and were hard in character. Experiments were tried in which various amounts of sodium and calcium hydroxide were added to the water in which the slides were washed just prior to staining. Treatment with N/100 calcium hydroxide for 15 min. was found to give the most satisfactory results, although further experimentation is needed. When distilled water was used throughout the various processes it gave results that were as unsatisfactory as those obtained with the Aberystwyth tap water. Alvarez (1931) reports that distilled water in equilibrium with the air will have absorbed enough carbonic acid to lower its pH to 5.6-5.8 which is sufficiently acid to upset the staining reactions.

The effect of the pH of the medium on the staining reaction of chromatin has been known for many years, but it is a point that is seldom given consideration, since the water supply for most of the important cytological laboratories is on the alkaline side of neutrality and therefore most suitable for staining with basic dyes.

The pollen of all the plants was examined, and the percentage of normal pollen was determined just prior to the exertion of the anthers. Three anthers from different parts of the inflorescence were placed in a drop of aceto-carmin. These were cut into small pieces and the pollen grains gently forced out with the flat side of a scalpel. The counts were determined immediately under the low power of a microscope. In the majority of the plants the bad pollen was easily recognised, since it was completely empty of nuclear contents. In some plants, however, some of the pollen had evidently commenced degeneration at a later stage. These pollen grains were not completely empty at this time, but their unhealthy appearance, as shown by their low staining capacity, indicated that they were degenerating. Approximately 300 pollen grains were counted on each of the three plantlets which were originally clonal cuttings off the same plant. Since these plantlets had been exposed to slightly different environmental conditions throughout the winter, it was felt that the average of the total of these three counts should give fairly reliable data.

The accompanying figures were drawn with the aid of a Zeiss camera-lucida, using a Zeiss apo. 1.5 mm. objective in combination with compensating eyepieces of various magnifications. The $\times 30$ eyepiece was most frequently used and this gave magnifications of $\times 6200$ at bench level.

The microphotographs were taken by Mr H. C. Osterstock with apparatus of his own construction.

RESULTS.

- (i) *L. perenne* (bA-67) $n = 7 \times F. pratensis (bF-3) $n = 7$.$

The parent plants, a single F_1 plant (30-bE-1) and six back-crosses to *L. perenne* ♂ were available for study. The somatic chromosome number for all the plants is 14. It was hoped that some morphological characters of the chromosomes could be selected which would distinguish the parental complements. The measurements taken of the chromosome lengths in *L. perenne* (Text-fig. 29) show a range from 3.4 to 5.1 μ , while those of *F. pratensis* (Text-fig. 9) range from 3.8 to 6.6 μ . Such a slight difference is of no significance, since we may expect a variation of this order in different cells of the same root tip. Mitosis in the F_1 and back-crosses is perfectly regular and indistinguishable from that in either of the parents. Constrictions are clearly visible in most of the chromosomes. The primary constriction is usually located at the point of flexure, and a short distance along one of the arms a very wide secondary constriction is often seen in

some of the chromosomes. This gap in the chromatin is frequently over 0.5μ wide, and in one chromosome in Text-fig. 10 it exceeds 1μ in width. This makes counting very difficult, especially when the condition is found in some of the other hybrids with high chromosome numbers. The occurrence of such gaps varies in *L. perenne* and *F. pratensis* from



Text-figs. 1-6. $\times 3100$.

Text-fig. 1. Heterotypic metaphase of *L. perenne* (bA-67).

Text-fig. 2. Heterotypic metaphase of *F. pratensis* (bF-3).

Text-fig. 3. Heterotypic metaphase of 30-bE-1 (F_1 of bA-67 \times bF-3).

Text-fig. 4. Diakinesis of 30-bE-1 (F_1 of bA-67 \times bF-3).

Text-fig. 5. Diplotene of 30-bE-1 (F_1 of bA-67 \times bF-3).

Text-fig. 6. Heterotypic metaphase of 71-bE-1 (back-cross of 30-bE-1 to *L. perenne*).

The total number of chiasmata and the number of terminal chiasmata are given for each figure.

two to seven per nucleus, although with good fixation a constriction is generally visible in all the chromosomes.

It is often very difficult to see the connections bridging these gaps, since they are evidently achromatic. Attempts were made to determine their nature by using various combinations of acid stains with Heidenhain's haematoxylin. It was possible to get satisfactory preparations with the achromatic material in the cell nicely contrasted, but even under such conditions no definite connecting structure could be observed. With

gentian violet it is sometimes possible to see two faint connecting strands in the narrow gaps, so in all the illustrations these connections are shown whether they are visible or not.

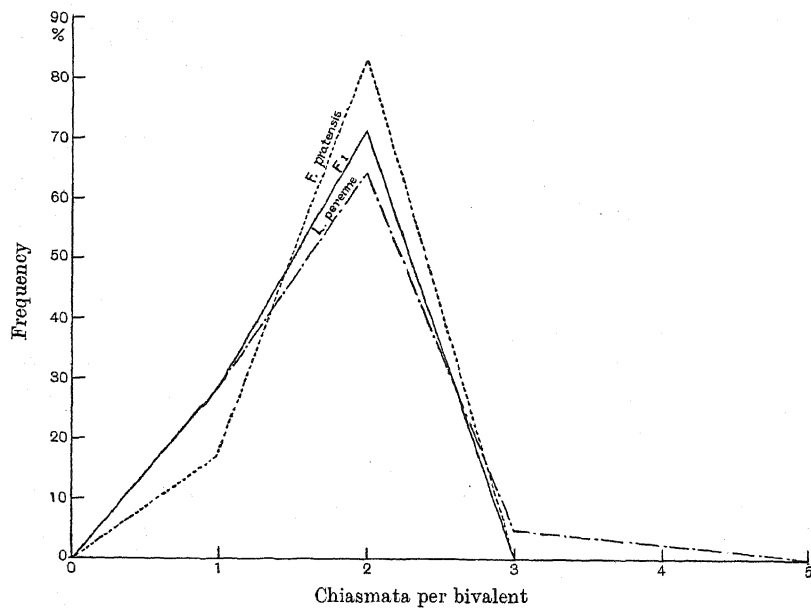
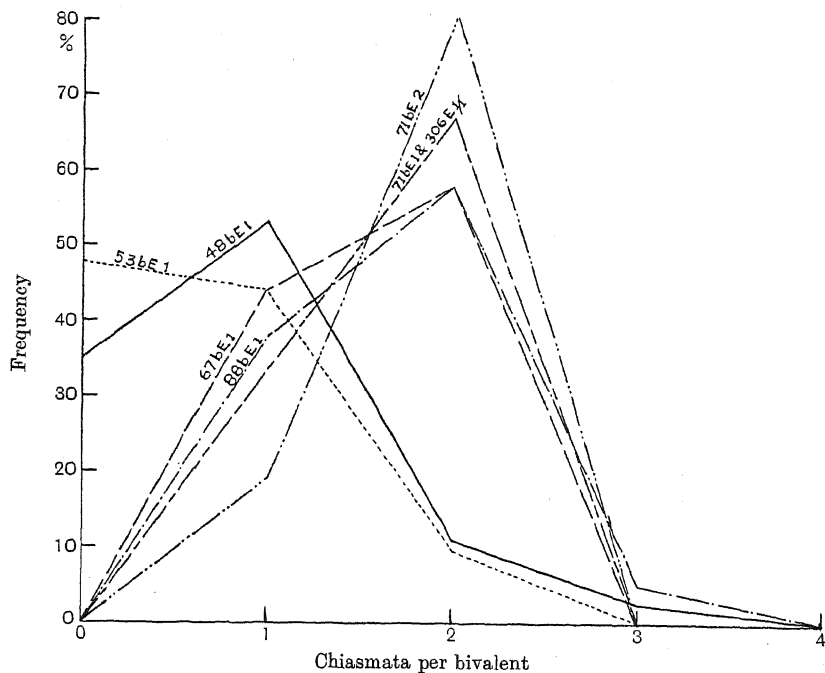
The meiotic behaviour in the parent plants *L. perenne* and *F. pratensis* is very similar, and no irregularities in either have been observed. Text-figs. 1 and 2 show side views of heterotypic metaphase plates. The majority of the bivalents are in the form of rings with two terminal or occasionally sub-terminal chiasmata. Interstitial chiasmata are never observed at this stage. This is presumably the result of considerable terminalisation between diplotene and metaphase. Various classes of data regarding these species and their progeny have been collected and recorded in Table I. The chiasma frequency per bivalent is very similar,

TABLE I.

	Per- centage good pollen	Meta- phase uni- valents	Meta- phase bivalents %	Terminal- isation coeffi- cient	Chiasma frequency per bivalent
Parents					
♀ <i>L. perenne</i> (bA-67)	92	—	100	0.64	1.81
♂ <i>F. pratensis</i> (bF-3)	81	—	100	0.93	1.88
<i>F</i> ₁					
30-bE-1	13	—	100	0.66	1.71
Back-crosses to <i>Lolium</i> ♂					
88-bE-1	92	—	100	0.71	1.66
30-bE-1/1	90	—	100	0.81	1.66
30-bE-1/2	80	—	—	—	—
67-bE-1	84	—	100	0.77	1.57
71-bE-1	77	—	100	0.60	1.66
71-bE-2	85	—	100	0.80	1.80
48-bE-1	40	34.5	65.5	0.50	0.80
53-bE-1	0	31.2	68.8	0.53	0.62

being 1.81 for *L. perenne* and 1.88 for *F. pratensis*. The latter species has a higher terminalisation coefficient, but this is of doubtful significance since all the chiasmata are so nearly terminalised.

The homologous chromosomes of the *F*₁ plant 30-bE-1 regularly form bivalents. The types of metaphase configurations are very similar to both the parents, and the chiasma frequency is not significantly lower. The polygons in Text-fig. 7 illustrate the similarity between the parents and *F*₁ in chiasma formation. Forty-two bivalents (six complete nuclei) of each plant were examined. Complete statistical evidence was not calculated for the earlier stages, but Text-figs. 4 and 5 are typical of several observed. In the late diplotene (Text-fig. 5) there are a number of interstitial chiasmata, the terminalisation coefficient being only 0.33.

Text-fig. 7. Polygons of chiasma frequencies in *L. perenne*, *F. pratensis* and the F_1 (30-bE-1).Text-fig. 8. Polygons of chiasma frequencies in seven back-crosses of 30-bE-1 to *L. perenne*.

This is increased to 0.53 at diakinesis, while at metaphase this coefficient averages 0.66. At this stage no interstitial chiasmata have been observed.

The only other study of chiasma frequency in the Gramineae is that of Darlington (1931 *a*) on *Triticum* hybrids. His configurations of *T. monococcum*, *T. aegilopoides* and F_1 are strikingly similar to those illustrated here. He also finds a very slight reduction in chiasma frequency in the F_1 .

Only 13 per cent. good pollen is produced by the F_1 plant 30-bE-1 in spite of normal prophase pairing, and it is not known whether any of this pollen is viable. Certainly under actual breeding conditions it will have little chance of reaching an ovule, since the anthers fail to dehisce normally. The first signs of degeneration are seen after tetrad formation. The tapetal cells appear to degenerate sooner than is usual, and development in most of the microspores appears to cease.

The data collected on seven back-crosses to various plants of *L. perenne* are very interesting. In five of these plants bivalents are regularly formed, and the chiasma frequency is not significantly lower than either of the parents, plant 71-bE-2 having a chiasma frequency equal to that of *L. perenne*. In contrast to this, plants 48-bE-1 and 53-bE-1 have about one-third of their chromosomes unpaired at metaphase and their chiasma frequency is 0.80 and 0.62 respectively, or one-half to one-third of that found in the other back-cross plants. This condition is illustrated in Text-fig. 8. Four of the polygons are very similar, having their mode at two chiasmata per bivalent, while plant 48-bE-1 has its modal class at one chiasma, and 53-bE-1 at less than one. The difference between these two groups is sufficiently marked to be significant. This point will be considered later in the light of additional evidence. Text-fig. 6 illustrates the type of metaphase pairing that is common to the group of back-crosses with the high chiasma frequency, while Text-fig. 20 shows a typical metaphase in plant 48-bE-1 with the low chiasma frequency. There are four univalents in the latter cell, and four of the five bivalents have only one chiasma each.

Meiosis in plant 48-bE-1 was carefully observed from heterotypic metaphase until tetrad formation. The bivalents separate normally at the equatorial plate while the univalents lie scattered throughout the cell. During anaphase most of the univalents come to the equatorial plate and split equationally as in Text-fig. 23. One of the dividing univalents is seen lying near one of the poles, and both of these chromatids would presumably be included in the same daughter nucleus. The chromatids usually separate, but often fail to reach the poles in time to

be included in the daughter nuclei. These can be observed throughout interkinesis as particles of chromatin lying separate from the daughter nuclei. They are, however, again included in the homotypic division, and during anaphase and telophase can be seen segregating at random. Many of the resulting pollen grains must receive a genetically unbalanced complement of chromosomes. It is therefore not surprising that only 40 per cent. of the pollen appears to be normal.

- (ii) *F. pratensis* (bF-2) $2n = 14 \times F_1$ of *L. perenne* \times *L. perenne* var. *multiflorum* (21-bE-1) $2n = 14$.

This cross closely approximates to the reciprocal of the *L. perenne*-*F. pratensis* hybrid discussed above. The fact that the male parent is an F_1 of an intervarietal cross need not complicate matters, since Jenkin (1924, 1931 *b*) reports that these two varieties are closely related genetically. He found the hybrid plants to be quite as self-fertile as either of the parental varieties. In spite of these observations it was found that the male parent in the present cross (21-bE-1) produced much less good pollen than the *F. pratensis* plant used as the female (Table II).

TABLE II.

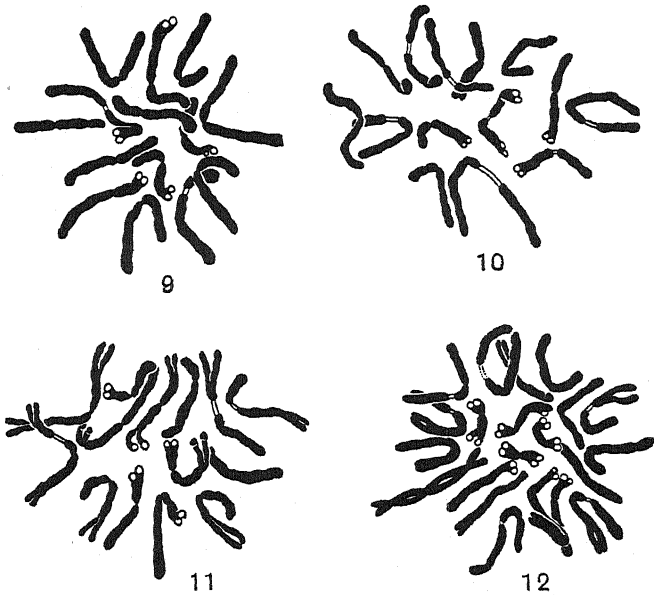
	Percentage					Terminal- isation coeffi- cient	Chiasma frequency per bivalent
	Good pollen	Uni- valents	Bi- valents	Tri- valents	Quadri- valents		
Parents							
♀ <i>F. pratensis</i> (bF-2)	87	—	100	—	—	1.00	1.59
♂ 21-bE-1*	25	—	100	—	—	0.73	1.59
<i>F</i> ₁							
17-bI-1	0	2	98	—	—	0.66	1.61
17-bI-2	0	—	100	—	—	0.71	1.57
17-bI-4	0	2.4	92.8	—	4.8	0.88	1.62
17-bI-6	0	—	100	—	—	0.83	1.62
Back-cross of 17-bI-6 to <i>L. perenne</i>							
56-bE-1	61	—	100	—	—	0.81	1.50
56-bE-2 (triploid)	41	7	57	36	—	0.84	—
56-bE-3	74	—	100	—	—	0.72	1.76
Back-cross of 17-bI-6 to <i>F. pratensis</i>							
44-bI-1	0	—	—	—	—	—	—
56-bE-3 selfed	—	—	—	—	—	—	—
56-bE-3/1	37	—	100	—	—	0.63	1.43
56-bE-3/2	23	33.3	66.6	—	—	0.78	0.88

* 21-bE-1 is an F_1 of *L. perenne* $n = 7 \times$ *L. multiflorum* $n = 7$.

Somatic plates of the parents and F_1 are shown in Text-figs. 9, 10

and 11. No constant morphological differences could be observed between the chromosomes of these plants.

The data collected from meiotic studies are tabulated in Table II. The chiasma frequency in the four F_1 plants is remarkably constant, varying only from 1.57 to 1.62 chiasmata per bivalent. Two polygons were drawn (Text-fig. 21) which compare the chiasma frequency of these plants with their parents. These correspond closely throughout their



Text-figs. 9-12. $\times 3100$.

Text-fig. 9. Metaphase in root tip of *F. pratensis* (bF-2).

Text-fig. 10. Metaphase in root tip of F_1 of *L. perenne* \times *L. perenne* var. *multiflorum* (21-bE-1).

Text-fig. 11. Metaphase in root tip of 17-bI-1 (F_1 of bF-2 \times 21-bE-1).

Text-fig. 12. Metaphase in root tip of triploid plant 56-bE-2 (back-cross of 17-bI-6 to *L. perenne*).

entire length. We can conclude from this that the effect of hybridity on chiasma frequency in this particular case is nil. Although prophase pairing is generally normal, a small proportion of univalents was observed in 17-bI-1 and 17-bI-4.

A chain of four chromosomes is shown at metaphase in Text-fig. 15. A microphotograph was taken of the same cell (Plate VII, fig. 7). At diakinesis a slightly different type of configuration was seen (Text-fig. 17). Here a rod-shaped bivalent is attached to a ring bivalent by a



Text-figs. 13-20.

Text-fig. 13. Heterotypic metaphase of *F. pratensis* (bF-2).

Text-fig. 14. Heterotypic metaphase of 21-bE-1.

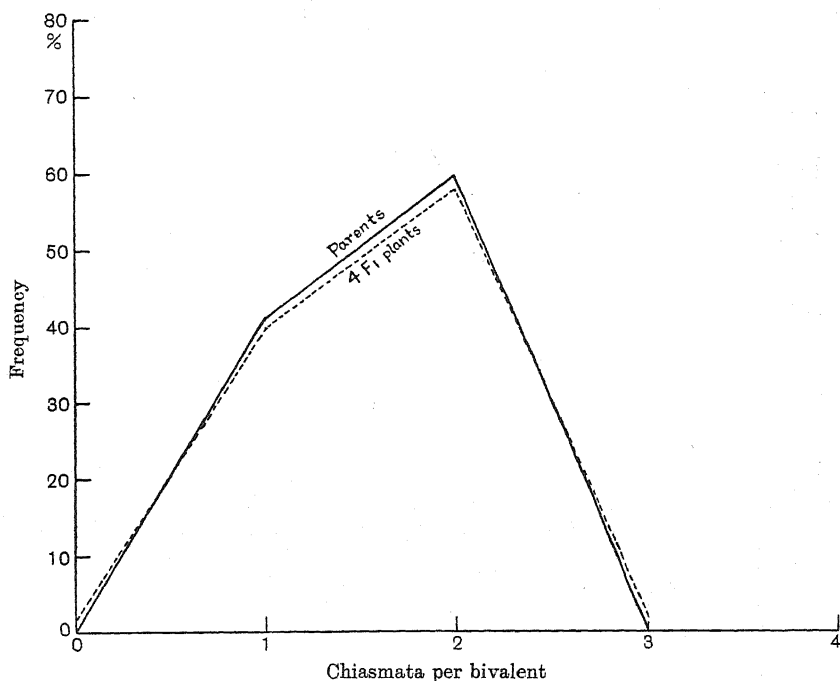
Text-fig. 15. Heterotypic metaphase of 17-bI-1 (F_1 of bF-2 \times 21-bE-1).Text-fig. 16. Heterotypic metaphase of 17-bI-6 (F_1 of bF-2 \times 21-bE-1).Text-fig. 17. Diakinesis of 17-bI-4 (F_1 of bF-2 \times 21-bE-1).Text-fig. 18. Heterotypic metaphase of 56-bE-2, $2n=21$.

Text-fig. 19. Heterotypic metaphase of 56-bE-3/2.

Text-fig. 20. Heterotypic metaphase of 48-bE-1.

Text-fig. 13, $\times 2100$. Text-figs. 14-20, $\times 3100$.

terminal chiasma. To obtain configurations of this kind in a diploid there must have been pairing between identical parts in chromosomes which were non-homologous for the remainder of their length. This indicates that translocation or segmental interchange has occurred between non-homologous chromosomes in one of the parental species at some time during their differentiation.



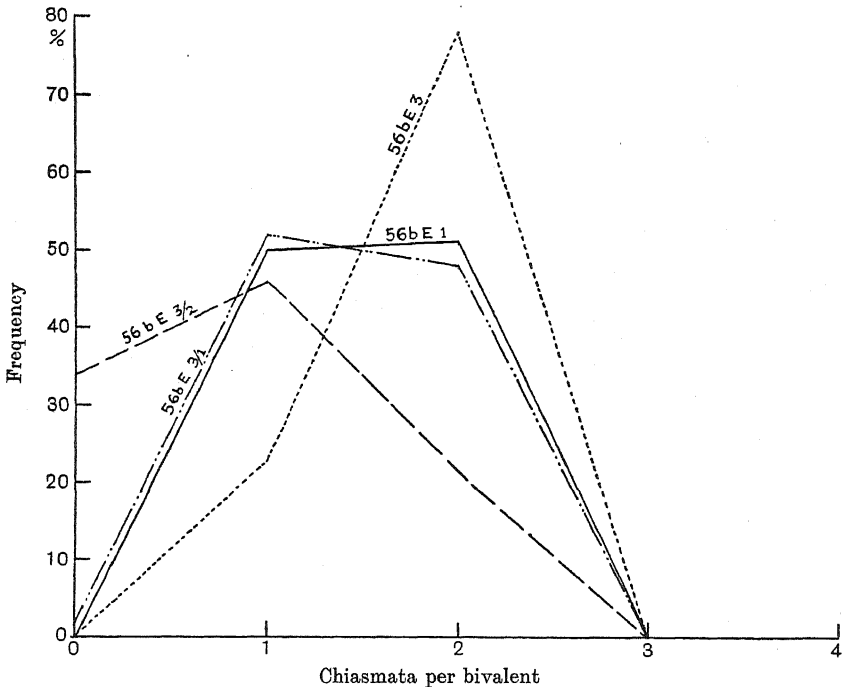
Text-fig. 21. Polygons of chiasma frequencies in the parents bF-2, 21-bE-1 and four F_1 plants.

Interlocking of bivalents was observed in plant 17-bI-6 (Text-fig. 16). It must be rare, since this is the only time it has ever been observed in any species of *Festuca* or *Lolium*. Darlington (1931 a) reports having seen it once in *T. aegilopoides*. It has, however, been frequently observed in *Oenothera* by Catcheside (1931) and in *Campanula* by Gairdner and Darlington (1931).

The failure of the F_1 plants to produce any good pollen cannot be the direct result of any observed irregularities up to tetrad formation, but is due to degeneration between tetrad formation and pollen maturity.

Of three plants from a back-cross to *L. perenne*, one (56-bE-2) was found to be a triploid, and had most probably originated by the fertili-

sation of an unreduced female gamete. The pairing in this plant is very interesting; the proportions of univalent, bivalent and trivalent configurations are given in Table II. The maximum number of bivalent and multivalent configurations should be seven in a triploid of this kind, but a number of plates were found which had more than this number. One of these is shown in Text-fig. 18, in which there are nine configurations, six of them being bivalents and three trivalents. This phenomenon is presumably due to the same cause which resulted in the formation of

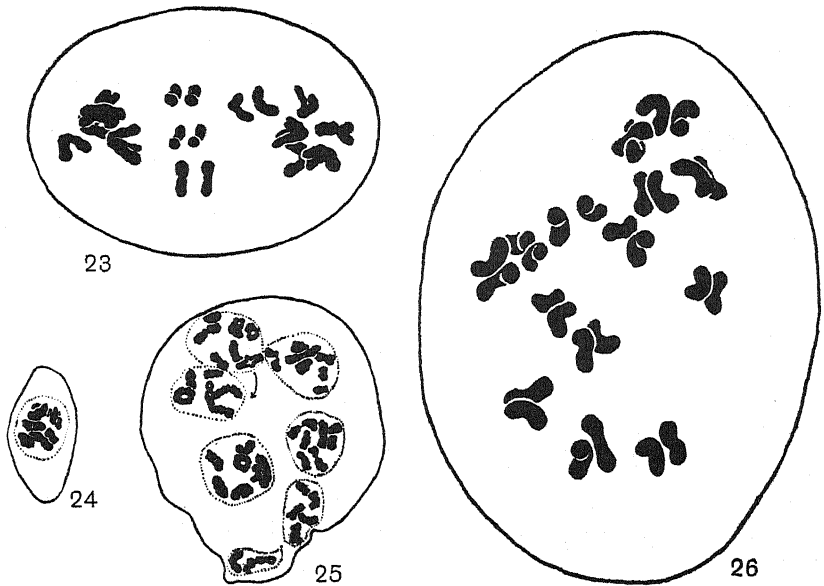


Text-fig. 22. Polygons of chiasma frequencies in four of the progeny plants of bF-2 \times 21-bE-1.

chains of four in the diploid plant 17-bI-4, and its occurrence might be expected since two of the chromosome sets in the triploid probably came from this plant.

Meiosis in the back-cross to *F. pratensis* is much more irregular than in the back-cross to *L. perenne*. Only one plant (44-bI-1) was available and it was found to produce no good pollen. The presence of abnormalities was variable in different florets and even in different parts of the same anther. In a limited number of instances pairing was complete, and development was normal till after tetrad formation. Many polynucleate

pollen mother cells were observed, some containing as many as six nuclei in a single cell. These resulted from a failure of wall formation in the last two or three somatic divisions of the sporogenous tissue. The functioning of the spindle has been normal in so far as the chromosomes have been separated into their respective nuclei. One of these giant cells is shown in Text-fig. 25, and in a microphotograph (Plate VII, fig. 9). All the nuclei are at diakinesis. In one of them the nuclear membrane and two chromosomes are separated from the remainder. This is probably the result of



Text-figs. 23-26.

Text-fig. 23. Heterotypic anaphase of 56-bE-3/2. $\times 3100$.

Text-fig. 24. Normal pollen mother cell in 44-bI-1. $\times 1050$.

Text-fig. 25. Multinucleate pollen mother cell in 44-bI-1. $\times 1050$.

Text-fig. 26. Tetraploid microspore in diploid plant 44-bI-1. $\times 3100$.

damage during preparation since the cell wall is also distorted at this point. The adjacent figure 24 illustrates the size of a normal cell. The microphotograph (Plate VII, fig. 8) shows a locule of an anther which contains cells at various meiotic stages from prophase to dyad. This appears to be the result of degeneration having halted development at different stages.

Syndiploid metaphase plates with double the normal complement of bivalents were occasionally seen. These arose through the chromosomes of a binucleate pollen mother cell orientating themselves on a common

equatorial plate. Failure in wall formation may also take place at any time throughout the meiotic divisions. Normally a cell wall is formed between the dyad nuclei, but under abnormal conditions there may be a partial cleavage in the cytoplasm, or the chromosomes may be distributed to the poles without any apparent attempt at wall formation. The cell drawn in Text-fig. 26 has the tetraploid chromosome number. The bivalents have separated from one another, and the chromosomes have also divided equationally, but are still lying close together. In the heterotypic division there was evidently a partial failure of the spindle coupled with complete failure of wall formation. The chromosomes had split in the homotypic division without any apparent orientation on the equatorial plate or any subsequent movement of the chromosomes to the daughter nuclei. This failure of the spindle and wall formation is very similar to that found in *Kniphofia* by Moffett (1932). Numerous polyploid pollen grains are undoubtedly formed as the result of the above irregularities, but they all appear to degenerate before maturity.

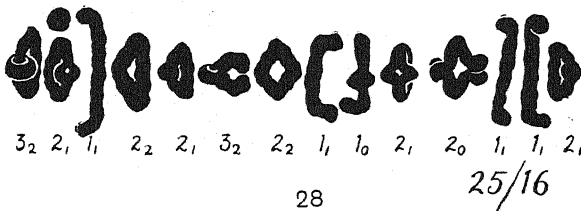
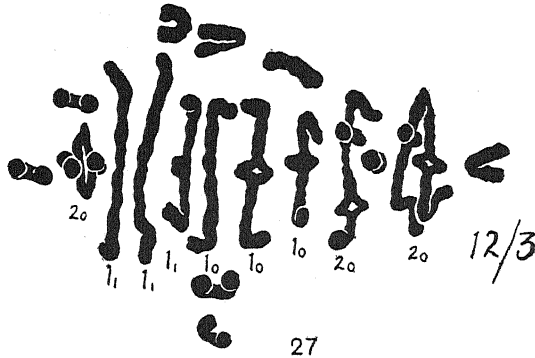
Plant 56-bE-3 was selfed and two of its progeny were studied. Pairing was normal in one plant, but in the other (56-bE-3/2) one-third of the chromosomes remained unpaired (Text-fig. 19). This behaviour is strikingly similar to that found in 48-bE-1 and 53-bE-1, and is probably best accounted for by assuming segregation of some genetic factor limiting chiasma formation. The polygons of chiasma frequency in Text-fig. 22 show how sharply 56-bE-3/2 differs from the remainder.

The F_1 (17-bI-4) was outcrossed to *F. arundinacea*, and two plants, 69-bI-1 and 2, were produced. Contrary to expectation both had 29 instead of 28 chromosomes. One very small chromosome was consistently observed during meiosis, so it is probable that the extra chromosome arose through fragmentation either in the male parent or the F_1 . The pairing in these two plants is very different.

	Univalents %	Bivalents %	Trivalents %	Chiasma frequency
69-bI-1	61.0	36.6	2.4	0.83
69-bI-2	22.2	71.4	6.4	1.54

There are three times as many univalents in 69-bI-1, and the chiasma frequency is half of that in 69-bI-2. Typical metaphase plates of these two plants are shown in Text-figs. 27 and 28. It is important to note in the figure with the low chiasma frequency that it has a much higher proportion of rod-shaped bivalents. This case is comparable with the three diploid hybrids in which a marked fall in chiasma frequency was observed, and the same genetical explanation should be valid.

It is important to note the double nature of the terminal chiasmata in the accompanying illustrations. The microphotograph in Plate VII, fig. 7, is particularly convincing in this respect. According to the chiasma theory of pairing (Darlington, 1929 *a*), the formation of terminal as well as interstitial chiasmata has involved an exchange of partner. If this is the case terminal chiasmata would always be expected to be double, as has been found in these studies.



Text-figs. 27 and 28. $\times 3100$.

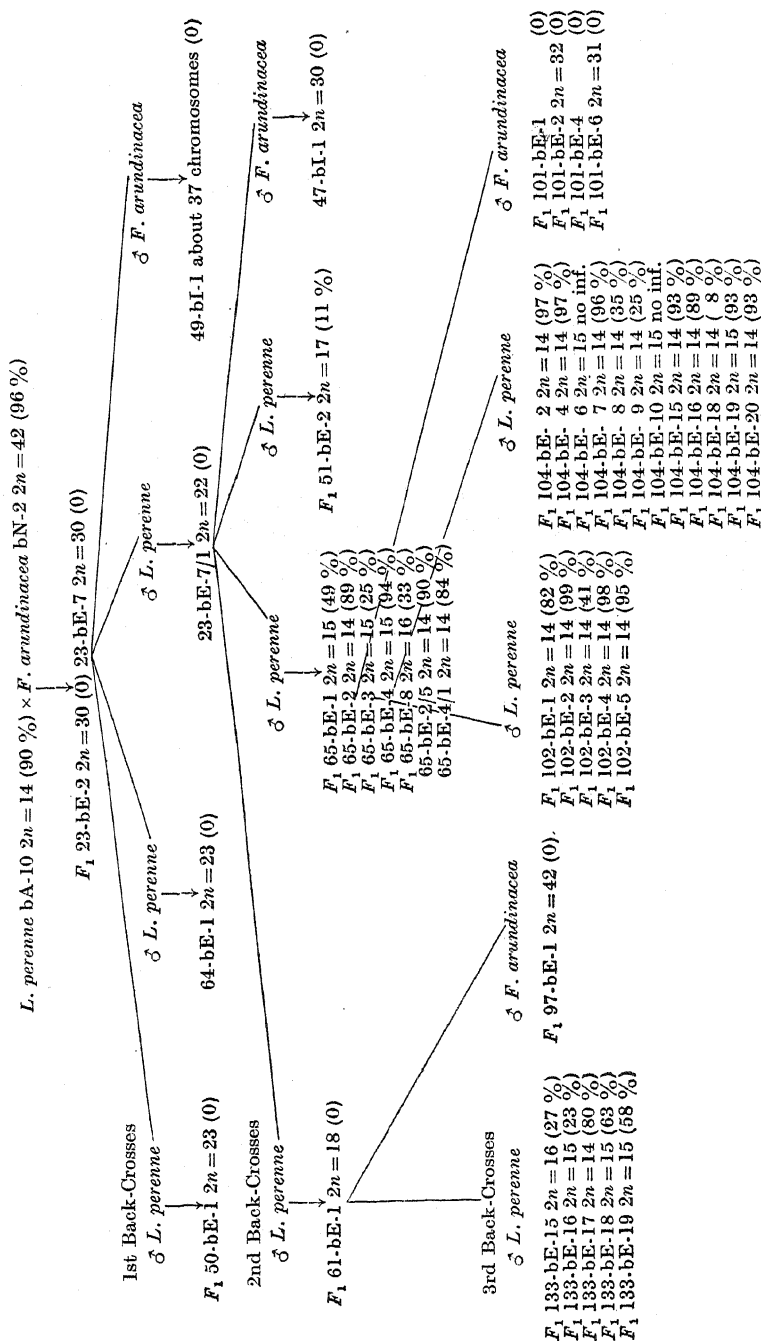
Text-fig. 27. Heterotypic metaphase of 69-bI-1, $2n=29$.

Text-fig. 28. Heterotypic metaphase of 69-bI-2, $2n=29$.

(iii) *L. perenne* (bA-10) $2n = 14 \times F. arundinacea$ (bN-2) $2n = 42$.

The complicated situation existing in this hybrid and its derivatives is indicated by the accompanying diagrammatic scheme (Table III), which gives the pedigree of the plants supplied by Mr Jenkin, their chromosome number and the percentage of good pollen which they produced. By repeated back-crossing to both of the parental species, a series of plants with chromosome numbers varying between 14 and 42 was obtained.

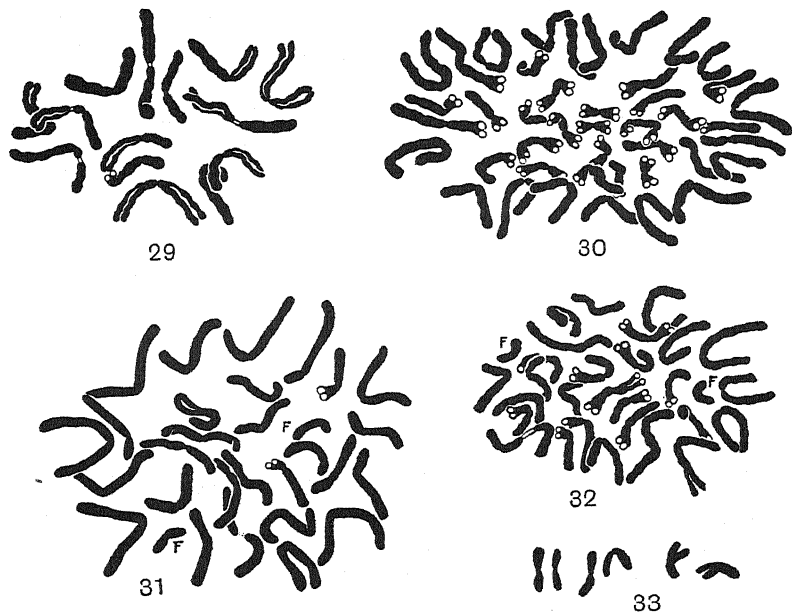
TABLE III.



N.B. The figures in brackets give the percentage of good pollen produced. The plant designations given are those in use at the Welsh Plant Breeding Station and give the cross number, type of cross, generation and the plant number.

The somatic chromosomes of the parental species are very similar in length and shape (Text-figs. 29 and 30), but the very wide constrictions or gaps which are so frequently seen in *L. perenne* are rare in *F. arundinacea*.

The types of metaphase configurations of parental species are very similar. *L. perenne* has an average chiasma frequency of 1.62 per bivalent for six nuclei, while the metaphase plate of *F. arundinacea* shown in



Text-figs. 29-33. $\times 3100$.

Text-fig. 29. Metaphase in root tip of *L. perenne* (bA-10) $2n=14$.

Text-fig. 30. Metaphase in root tip of *F. arundinacea* (bN-1) $2n=43$.

Text-fig. 31. Metaphase in root tip of 23-bE-7 (F_1 of bA-10 \times bN-2) $2n=30$.

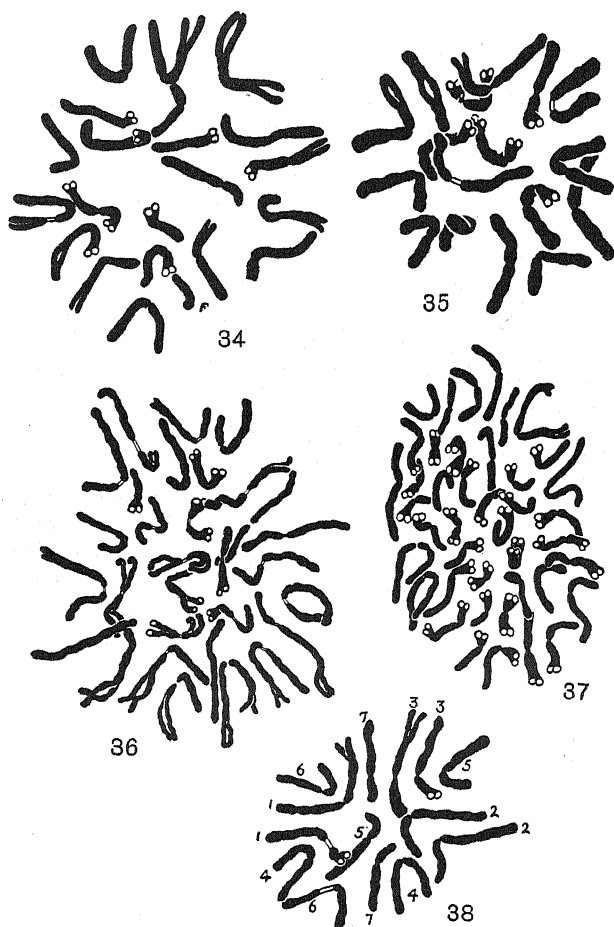
Text-fig. 32. Metaphase in root tip of 23-bE-2 (F_1 of bA-10 \times bN-2) $2n=30$.

Text-fig. 33. Small chromosomes or fragments from different cells in 23-bE-7.

Text-fig. 40 has a chiasma frequency of 1.5 per bivalent. Multiple associations have never been observed in either species.

Two F_1 plants (23-bE-2 and 23-bE-7) have been studied. Both of these plants have 30 instead of 28 chromosomes. These extra chromosomes have most probably originated through fragmentation. This is indicated by the fact that both of these chromosomes are much smaller than any of the others in these plants. These fragments are identified by the letter *F* in the somatic Text-figs. 31 and 32, and six are drawn

separately in Text-fig. 33. The chromosomes have probably been fragmented at some point which is structurally weak. The wide



Text-figs. 34-38. $\times 3100$.

Text-fig. 34. Metaphase in root tip of 23-bE-7/1, $2n=22$.

Text-fig. 35. Metaphase in root tip of 51-bE-2, $2n=17$.

Text-fig. 36. Metaphase in root tip of 47-bI-1, $2n=30$.

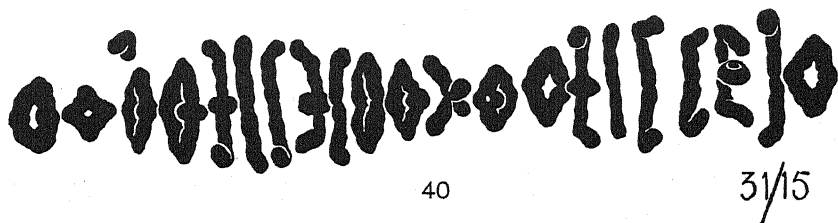
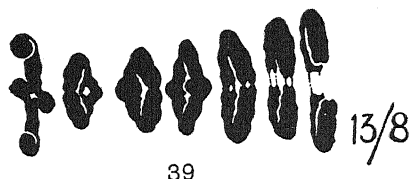
Text-fig. 37. Metaphase in root tip of 97-bE-1, $2n=42$.

Text-fig. 38. Metaphase in root tip of *F. loliacea*, $2n=14$.

secondary constrictions in certain chromosomes of *L. perenne* would appear to be a likely place for these breaks to occur. If this has taken place the segment with the attachment constrictions would be sufficiently

long to be indistinguishable, while the short arms would correspond very closely to the length of the observed fragments. The fragments in Text-fig. 33 show definite attachment constrictions, which suggests that they must be capable of developing constrictions immediately after fragmentation.

Meiosis was very similar in both F_1 plants, so only 23-bE-2 is discussed fully. A metaphase plate of 23-bE-7 is shown, however, in Text-fig. 41. On analysing the configurations in seven metaphase nuclei of 23-bE-2 it was found that 32.4 per cent. of the chromosomes remained



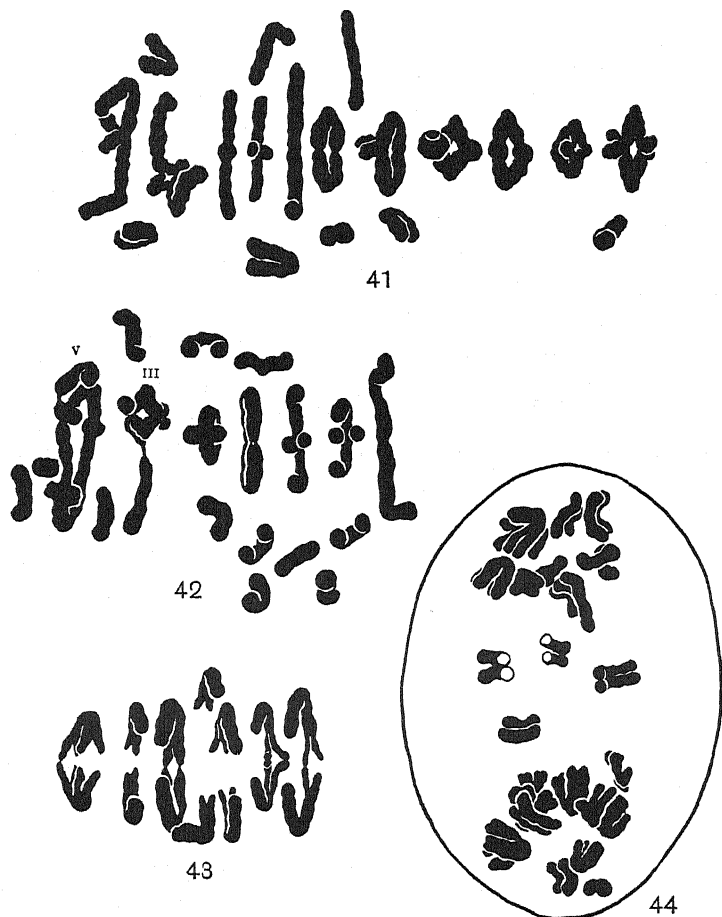
Text-figs. 39 and 40. $\times 3100$.

Text-fig. 39. Heterotypic metaphase of *L. perenne* (bA-10) $2n=14$.

Text-fig. 40. Heterotypic metaphase of *F. arundinacea* (bN-1) $2n=43$.

unpaired, 55.2 per cent. formed bivalents, 4.3 per cent. trivalents, 5.7 per cent. quadrivalents, and 2.4 per cent. quinquivalents. The occurrence of a configuration of the latter type is most unexpected, since there should be only four homologous chromosomes unless one of the two extra chromosomes is included in this configuration. The other possibility is that this is another instance of pairing between identical parts in otherwise non-homologous chromosomes. The presence of quadrivalent and quinquivalent configurations shows that there must be pairing between the *Festuca* and *Lolium* chromosomes as well as autosyndesis in the former. The metaphase plate in which the quinquivalent was found is drawn in Text-fig. 42. The early anaphase stage was particularly clear in one of the preparations. Text-fig. 43 shows the pulling apart of the

chromatids in some of the bivalents. The chromosome ends are undivided wherever terminalisation is complete, but in others the chromatids are separated back to the point where terminalisation of the chiasmata had



Text-figs. 41-44. $\times 3100$.

Text-fig. 41. Heterotypic metaphase of 23-bE-7 (F_1 of bA-10 \times bN-2) $2n=30$.

Text-fig. 42. Heterotypic metaphase of 23-bE-2 (F_1 of bA-10 \times bN-2) $2n=30$.

Text-fig. 43. Early heterotypic anaphase of part of the complement of 23-bE-2.

Text-fig. 44. Early heterotypic anaphase of 23-bE-7.

ceased. A cell in the same plant is shown at a slightly later stage in Text-fig. 44. The bivalents have divided normally and have reached the poles, and four univalents lagging at the equatorial plate have commenced to split equationally. Only a few of the univalents behave in this

manner, probably only those that happen to lie nearest the equatorial plate, or those which have reached the proper stage in development to allow the forces of orientation to act. The remainder would be included in the nuclei at interkinesis without splitting, and would divide normally in the subsequent division. The chromosomes which had divided equationally in the first division lag at the anaphase of the second division, and segregate at random without splitting. The majority of tetrads appeared quite normal, but in a few cells it was possible to see small fragments lying outside the nucleus. These are probably chromosomes which had lagged in the preceding division and had failed to reach the poles in time to be included in the nucleus.

Degeneration usually commences soon after the cells of the tetrad round off into pollen grains. The tapetal cells as well as the pollen grains commence to take on an unhealthy granular appearance. At a later stage the pollen has an asteroid appearance due to the collapse of the cell wall. The immediate cause of this degeneration appears to be the failure of development of the whole anther and its contents rather than the cumulative effect of the degeneration of individual microspores.

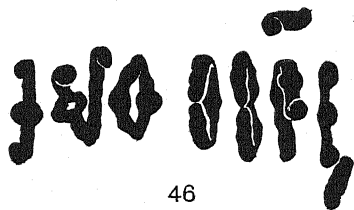
Plant 23-bE-7 was successfully used by Mr Jenkin as the female in back-crosses to both *L. perenne* and *F. arundinacea*. Three crosses of the former and one of the latter have been studied. Plant 23-bE-7/1 has 22 chromosomes (Text-fig. 34), while 50-bE-1 and 64-bE-1 each have 23 chromosomes. One fragment could be observed in each of these three plants. The metaphase configurations were very similar in all three plants. One of the plates in 23-bE-7/1 is illustrated in Text-fig. 45; it contains four trivalents, three bivalents and four univalents, one of the latter being a fragment. In this cell there is a higher proportion of trivalents than usual. The average for six nuclei is 26 per cent. univalents, 56 per cent. bivalents and 18 per cent. trivalents. The behaviour of the univalents is similar to that described in the F_1 . A tetrad containing micro-nuclei is shown in Text-fig. 48. These chromosome-like bodies are lying free in the cytoplasm, and are present in three of the four cells. Some are evidently the product of fusion of two chromosomes. One of these bodies is being segmented by cell-wall formation. This is very similar to an observation made on *Hyacinthus* by Darlington (1929 a, p. 31).

Degeneration of the anthers follows a somewhat similar course in these plants to that in the F_1 . Plant 50-bE-1 was observed more closely in this respect. The cell contents of the tetrads and tapetum stained darkly and had an unhealthy granular appearance. As this condition

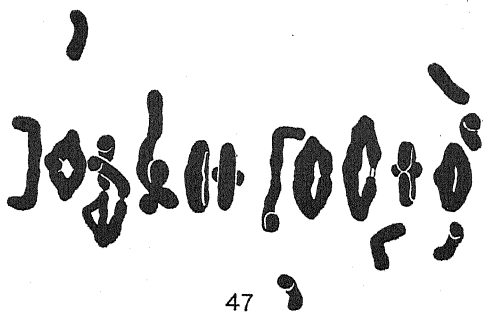
became more advanced, the tapetal cells separated from one another and lay scattered throughout the pollen sac amongst the degenerate tetrads. In other anthers, pollen was found at a slightly later stage in which the nuclear material had coagulated into small dense chromatic



45



46



47

Text-figs. 45-47. $\times 3100$.

Text-fig. 45. Heterotypic metaphase in 23-bE-7/1, $2n=22$.

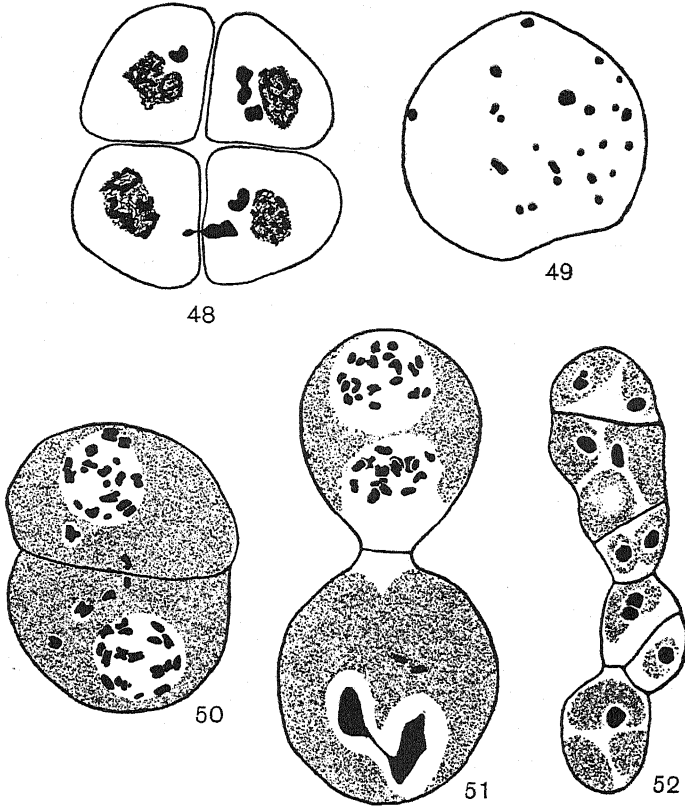
Text-fig. 46. Heterotypic metaphase in 61-bE-1, $2n=18$.

Text-fig. 47. Heterotypic metaphase in 47-bI-1, $2n=30$.

bodies. These were extremely variable in size, shape and number (Text-fig. 49). Sometimes collapsed pollen was observed which had the asteroid appearance similar to that found in 23-bE-2.

The four back-crosses to *F. arundinacea* (Table III, see crosses 49, 47, 97, 101) are all male sterile, and are particularly interesting on account of their abnormalities in anther development and meiotic

behaviour. It must be recognised that these crosses are not strictly comparable, since they belong to different generations and have a different chromosomal constitution. The anthers of 49-bI-1 were examined under



Text-figs. 48-52.

Text-fig. 48. Tetrad of 50-bE-1, showing chromosome being segmented by the cell wall. $\times 1050$.

Text-fig. 49. Degenerate pollen grain of 50-bE-1.

Text-fig. 50. Dyad in 49-bI-1. $\times 1050$.

Text-fig. 51. Two conjoined dyads in 49-bI-1. $\times 2100$.

Text-fig. 52. Multinucleate structure in the anther of 49-bI-1, resulting from incomplete separation of the pollen mother cells. $\times 550$.

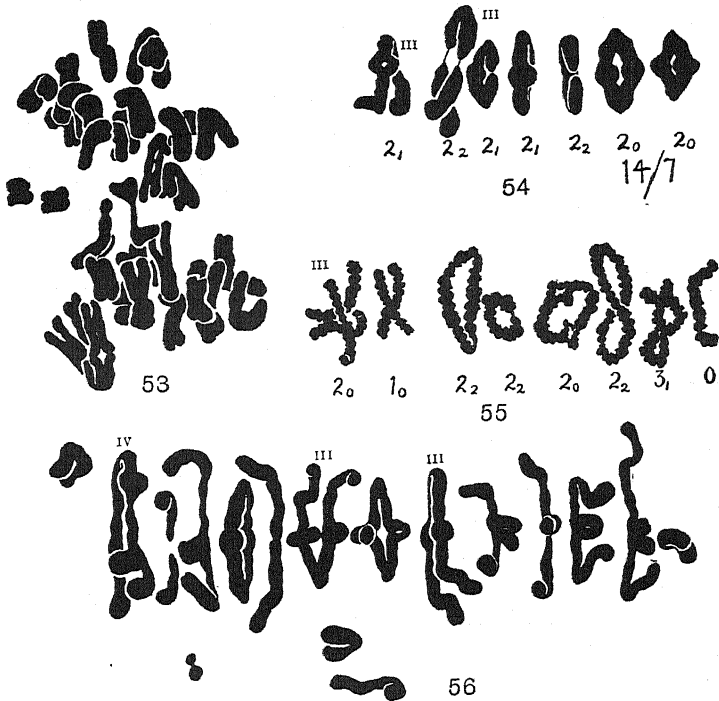
the binocular, and the commonest abnormality was the absence of one or two locules in each. Sometimes the anthers only developed into a peculiar looking prong. A picture of a typical floret is shown in Plate VII, fig. 6. Frequently only the basal part of the anther contained any sporogenous tissue. Normal tetrads or pollen grains are rarely produced,

since degeneration usually occurs at earlier stages. The first evidence of this is the failure of wall formation in the pollen mother cells. The appearance of this abnormality often gives the false impression that fusion of the cells has taken place (Text-fig. 52). In reality wall formation during the last few somatic divisions in the sporogenous tissue was incomplete; this resulted in the cohesion of a number of cells in the dyad and tetrad stage. Text-fig. 51 shows a somewhat similar condition. Development in wall formation was evidently arrested after a single septum had been formed between these two cells. The nucleus of the upper cell has accomplished the reduction division and the daughter nuclei are separated by their respective nuclear membranes, but there is no indication of the usual wall being formed. In the lower cell the two nuclei are still connected by a thin strand. At the top of this cell there is a definite cleavage in the cytoplasm, as if some stimulus to divide was operative. A more normal dyad is shown in Text-fig. 50 in which the nuclei are separated by a well-developed cell wall. A number of lagging chromosomes in this division have evidently failed to reach the poles in time to be included in the daughter nuclei.

Plant 47-bI-1 is much less abnormal than the above, although it has similar anther deficiencies. The somatic chromosome number is 30 (Text-fig. 36), and the meiotic pairing is fairly regular. In Text-fig. 47 there are two trivalents, nine bivalents and six univalents at the heterotypic metaphase. The meiotic divisions are very similar to that described for the F_1 plants. Immature pollen grains were observed which were regular in size and shape, but these all degenerated before maturity.

97-bE-1 is the progeny of a back-cross of 61-bE-1, $2n = 18$, with *F. arundinacea*, $2n = 42$. It was surprising to find that this plant had 42 somatic chromosomes. The fertilisation of an unreduced female gamete would be expected to give 39 chromosomes ($18 + 21$) in the resulting plant. Even if this had occurred it would still be necessary to account for the presence of three extra chromosomes. It is possible that these chromosomes arose by fragmentation, but it is extremely difficult to identify definitely large fragments in a somatic complement of this size, since the arms of some of the chromosomes are always lying in a different plane from the remainder. However, certain of the lagging univalents in the first division appear, as in Text-fig. 53, to be much smaller than any of the other chromosomes. These three extra chromosomes could also have been produced during the formation of the unreduced female gamete through certain of the univalents splitting before the meiosis restitution nucleus was formed.

From three to five univalents lag and divide equationally in the first division in 97-bE-1 (Text-fig. 53), though all seem to reach the poles in time to be included in the resting nuclei. In the second division these chromosomes again lag and segregate at random, but a small proportion fails to be included in the tetrad nuclei and can be seen lying free in the



Text-figs. 53-56. $\times 3100$.

Text-fig. 53. Heterotypic anaphase of 97-bE-1, $2n=42$.

Text-fig. 54. Heterotypic metaphase of 133-bE-15, $2n=16$.

Text-fig. 55. Diplotene of 133-bE-15, $2n=16$.

Text-fig. 56. Heterotypic metaphase of 101-bE-2, $2n=32+1$ fragment.

cytoplasm. Degeneration after tetrad formation takes place as described for the F_1 plants.

There were four plants studied in back-cross 101. One of these plants, 101-bE-1, produced the most striking abnormalities in floral structure. Three supernumerary pistils often replaced the anthers. These were sometimes indistinguishable from the normal ovary except that they were in the position of the stamens. Flowers were dissected and examined at different stages in an endeavour to deduce the mode of

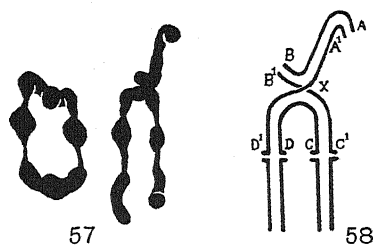
development of these abnormal structures. The different types of development appear to be as follows: sometimes the rudimentary bracts, which would normally develop into stamens, enlarge without further differentiation until they finally appear as bract-like structures about the size of the anthers. In other instances these bracts produce feathery stigmas at their tip—a sort of belated attempt at sex reversal. When there is an early differentiation towards the female condition in the rudimentary bracts, apparently normal supernumerary pistils are produced. A very few develop into abnormal anthers which degenerate at an early stage. Of the 65 florets that were dissected and critically examined under the binocular 40 had one or more supernumerary ovaries, and all but two had modified bracts, while none developed typical anthers. Microphotographs of these abnormalities are shown in Plate VII, figs. 2-5. It is of interest to note that only one plant in this family had these abnormalities. The other three all developed anthers in which the tetrads degenerated in the usual manner for this type of cross. These morphological differences cannot be the result of environmental differences, since these plants were produced under very similar conditions.

The mother plant of cross 101 has 14 somatic chromosomes, so that the progeny in a back-cross with *F. arundinacea* would be expected to have 28 chromosomes, but as in previous cases the chromosome number was higher. Plant 101-bE-2 had 32 and 101-bE-6 had 31 chromosomes. A metaphase plate of the former is shown in Text-fig. 56 which contains one quadrivalent, two trivalents, nine bivalents, four univalents and one very small fragment. The two quadrivalents in Text-fig. 57 are unusual, one forms a ring of four, while the other has a chiasma uniting the median portion of one chromosome with the sub-terminal portion of the other. The latter indicates that there must be homologous portions in different loci in these two chromosomes. An observation such as this provides a demonstration of genetical crossing-over.

If a chiasma or change of partner occurs at X (Text-fig. 58), as the chiasma theory of pairing demands, then the constitution of the four chromatids concerned must be AB , A^1D^1 , C^1B^1 and CD . B^1C^1 is shorter than any of the others and lacks an attachment constriction, while A^1D^1 is much longer than any of the others and contains two attachment constrictions. Since the four chromatids originate from two somatic chromosomes, only two types of chromatids could occur if no crossing-over had taken place. Therefore the unequal chromatids A^1D^1 and B^1C^1 can only have arisen as the result of crossing-over between the original chromatids which must have been A^1B^1 and C^1D^1 . It is obvious that this

type of irregular pairing could originate structural changes if the plant were fertile.

In *Oenothera* (Darlington, 1931 *b*) and *Pisum* (Sansome, 1932) similar proofs of crossing-over were deduced by observations of interstitial chiasma in the multiple rings of interchange heterozygotes. In both these cases the identity of the chromatids involved was inferred by their



Text-figs. 57 and 58.

Text-fig. 57. Two types of quadrivalents of 101-bE-4. $\times 3100$.

Text-fig. 58. Diagram of the chromatids in one of the quadrivalents.

TABLE IV.

Plant number	Chromosome number	Good pollen %	Metaphase configurations		
			Univalents %	Bivalents %	Trivalents %
65-bE-2	14	89	—	100	—
133-bE-17	14	80	—	100	—
Average 84.5					
65-bE-1	15	49	4.4	88.9	6.7
65-bE-3	15	25	3.4	86.6	10.0
65-bE-4	15	94	5.0	90.0	5.0
133-bE-16	15	23	2.3	84.4	13.3
133-bE-18	15	63	3.4	86.6	10.0
133-bE-19	15	58	3.4	86.6	10.0
Average 52					
65-bE-8	16	33	7.3	77.1	15.6
133-bE-15	16	27	6.2	75.0	18.8
Average 30					
51-bE-1	17	11	13.7	74.5	11.8
61-bE-1	18	0	15.8	64.8	19.4

terminal associations with other chromosomes in the rings, while in the above hybrid their identity is directly observable from their morphological differences.

The progeny of the second and third back-crosses to *L. perenne* had chromosome numbers ranging from 14 to 18. Meiosis in these crosses was carefully observed and six nuclei in each plant were analysed. These are grouped according to their chromosome number.

The pairing as shown by the above table is very regular. In all the

plants the maximum number of seven associations was attained. In the plants with 14 chromosomes the chiasma frequency is very similar to that calculated for *L. perenne*, and was 1.71 per bivalent for plant 133-bE-17. In the plants with more than 14 chromosomes the extra ones were either left unpaired or formed trivalents. The diplotene stage in the 16-chromosome plant is drawn in Text-fig. 55. It is interesting to note the position of the two chiasmata in the trivalent association; both are interstitial and probably on different sides of the attachment constriction, so that with further terminalisation they would take the form of one of the trivalents shown at metaphase in Text-fig. 54. There was nothing unusual in the chromosome behaviour in these crosses during the two meiotic divisions. When univalents were present they divided equationally in the first division and segregated at random in the second. Occasionally some of the lagging chromosomes in the second division would be excluded from the tetrad nuclei, but in spite of this a high proportion of healthy looking tetrads was formed.

It would be expected from the above observations that any decrease in the percentage of good pollen would be directly proportional to any increase in the number of chromosomes above 14. This is true on the whole according to the data given in the above table, but there are very marked exceptions. There is one plant with 15 chromosomes which produced 94 per cent. good pollen while the remainder varied from 23 to 63 per cent. This difference is in no way indicated by the pairing at metaphase, which only affords a very crude measure of their genetic homology. These results indicate that extreme caution should be exercised in making deductions regarding genetic homology from metaphase pairing. If chromosomes are paired at metaphase it may only mean that they are genetically homologous throughout a *portion* of their total length, and there is every reason to believe that the chromosomes in these complex hybrids must have undergone structural changes, *e.g.* pairing between *Festuca* and *Lolium* chromosomes which are only partially homologous would initiate translocation and segmental interchange, and segregation of these would result in deficiencies and re-duplication of parts of chromosomes. Even in the hybrid derivatives with the complete diploid complement in which bivalents are regularly formed, there might frequently be irregular segregation of parts of chromosomes which would result in genetically unbalanced gametes. When these factors are considered, the variation in the percentage of good pollen observed in plants with the same chromosome number is readily understandable.

A similar variation in the percentage of good pollen was observed in the 14 chromosome plants of crosses 102 and 104. Eleven of these plants averaged 94 per cent. while four plants averaged 27 per cent. The pollen mother cell material was not collected on all these plants, so it is impossible to state whether or not those with the low pollen fertility had faulty pairing.

In those plants which produced a high proportion of bad pollen, it was usually possible to see indications of precocious degeneration in the tapetal cells as well as in the immature pollen. In such cases the direct cause is probably the failure in the sporophytic generation rather than the cumulative effect of the degeneration of genetically unbalanced pollen grains. Under such conditions any small proportion of the pollen grains which happened to receive a genetically balanced complement would have little chance of survival.

In those plants which produced only a small proportion of bad pollen, the development in the sporophytic generation appears to be normal. The direct cause of degeneration in such cases is probably the genetically unbalanced condition in the individual pollen grains.

(iv) *Supposed natural hybrids.*

Festuca loliacea Huds. is considered to be a natural hybrid between *F. pratensis* and *L. perenne*. This conclusion has previously been based solely on ecological and taxonomic relationships (Ascherson and Graebner, 1902). Mr Jenkin has been studying the breeding behaviour of this species and he suggested that its cytology might prove interesting.

Two of the plants studied proved to be triploids. One of them (Bx-54) was collected north of Brentford, Middlesex, by Mr C. E. Hubbard of the Royal Botanic Gardens, Kew, and the other (Ba-174) by Prof. R. G. Stapledon from a fattening pasture at Castle Eaton, Wiltshire. Ba-174 has been used as the female in a cross with *L. perenne*, and a single F_1 plant (58-bE-1) was investigated which had 20 chromosomes. In the summer of 1931 Mr Jenkin collected a number of plants near Oxford which he has provisionally identified as *F. loliacea*. The chromosome number has been determined for ten of these and they all had 14 somatic chromosomes which appear to be very similar to those in *L. perenne* or *F. pratensis*, both in the lengths of the various pairs and in the position of the attachment and secondary constrictions (Text-fig. 38).

Meiosis has been studied in the two triploid plants as well as in the daughter plant with 20 chromosomes. A typical metaphase in Ba-174 is illustrated in Text-fig. 60, in which there are four univalents, four

bivalents and three trivalents. The results from the analysis of six metaphase nuclei in each plant are given below:

Plant number	Chromosome number	Metaphase configurations		
		Univalents %	Bivalents %	Trivalents %
Ba-174	21	38.1	41.3	20.6
Bx-54	21	25.6	51.1	23.3
58-bE-1	20	8.4	28.5	63.0

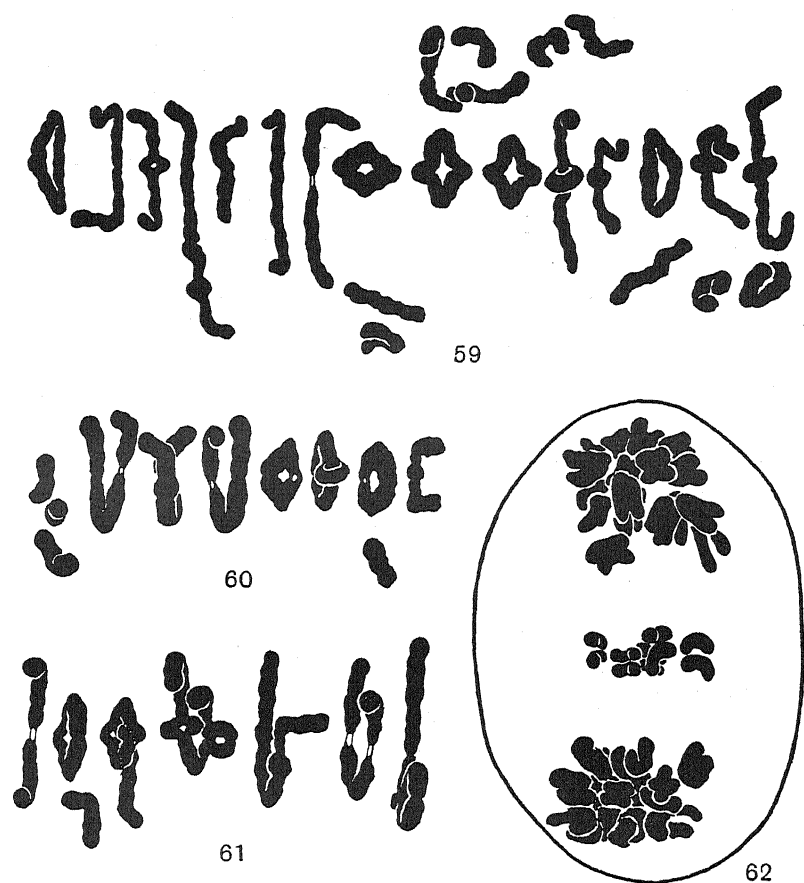
The maximum number of seven configurations was always observed at the metaphase of the first division. This was never exceeded as was occasionally found in the triploid plant 56-bE-2.

It will be noticed that 58-bE-1 has a much higher percentage of trivalents and fewer univalents than either of the other plants. The chromosomes of the trivalents in Ba-174 and Bx-54 are usually united in chains by two terminal or subterminal chiasmata, while in 58-bE-1 the frying-pan type of configuration and types with interstitial chiasmata are more common. A typical plate of the latter is shown in Text-fig. 61. The increase in this plant in the chiasma frequency and the percentage of chromosomes paired over that of the female parent, indicates that its complements must be more completely homologous.

There are two possible ways in which these *F. loliacea* plants could have originated. The first is that they are the progeny of an intergeneric hybrid between *L. perenne*, $n = 7$ and *F. pratensis*, $n = 7$. Mr Jenkin has artificially crossed these species, but only with considerable difficulty. Nevertheless there is no reason to doubt that it could occur occasionally in nature. It seems therefore most probable that the diploid *F. loliacea* plants arose in this manner. The triploids could also result from the same natural hybrid through the occasional fertilisation of unreduced gametes. This explanation appears to be very feasible, especially since a triploid (56-bE-2) was produced by Mr Jenkin by back-crossing a diploid F_1 of a *Festuca-Lolium* hybrid with *L. perenne*.

The second possible mode of origin of these triploid *F. loliacea* plants is that they are derivatives of a natural hybrid between *L. perenne*, $n = 7$ and *F. arundinacea*, $n = 21$. When the F_1 of the artificial hybrid between these species was back-crossed to *L. perenne*, it gave rise to plants with 22 and 23 chromosomes, one of which Mr Jenkin states to be very similar morphologically to the triploid *F. loliacea* plants. If these plants had originated in this manner it would be expected that a considerable variation in chromosome number between 14 and 28 would be found.

It will be necessary to study numerous *F. loliacea* plants collected from the same or similar plant associations to settle this point.



Text-figs. 59-62. $\times 3100$.

Text-fig. 59. Heterotypic metaphase in 93-bI-3 (F_1 of *F. gigantea* \times *F. arundinacea*), $2n=42$.

Text-fig. 60. Heterotypic metaphase of Ba-174, $2n=21$.

Text-fig. 61. Heterotypic metaphase of 58-bE-1, $2n=20$.

Text-fig. 62. Heterotypic anaphase of 93-bI-3.

(v) *F. gigantea* (Bn-81) $2n = 42 \times$ *F. arundinacea* (bN-1) $2n = 43$.

The male parent used in this cross contained a small chromosome or fragment in addition to the normal number of chromosomes. This extra chromosome is most readily detected in the pollen mother cells (Text-fig.

40), since it usually remains unpaired at metaphase, lags behind at the first division and divides equationally. All the pollen produced by this plant appeared to be normal, although the presence or absence of this extra chromosome might affect its viability. Definite somatic counts were obtained from four of the ten F_1 plants available, none of these, however, possessed the extra chromosome. This would be expected if the gametes containing the extra chromosome were incapable of fertilising *Festuca gigantea*.

The meiotic behaviour was determined in five plants, and these did not appear to differ in any respects. A typical metaphase plate is shown in Text-fig. 59 which contains one quadrivalent, fourteen bivalents and ten univalents. Trivalents are frequently seen in other cells. In twenty cells examined the number of univalents averaged 13.9 and varied from ten to nineteen. This indicates that there is fairly consistent failure of pairing between seven chromosomes from each parent. Autosynopsis must occur to some extent to account for the presence of quadrivalents, and this would also account for some of the variations in the number of univalents present. Some of the univalents lag at the heterotypic anaphase and divide equationally. In Text-fig. 62 there are five univalents lagging at the equatorial plate; this number is probably less than one-half of the total present in the cell. They always reach the poles in time to be included in the daughter nuclei. In the homotypic division these chromosomes segregate at random without splitting, and normal healthy-looking tetrads are formed. The usual symptoms of degeneration were observed between tetrad formation and maturity, and no good pollen was produced.

This cross is included in this study to permit of a comparison of the cytological evidences of hybridity between intergeneric and interspecific hybrids. If one were to judge genetic relationships solely from the pairing of the chromosomes at metaphase, one would be forced to the conclusion that *L. perenne* and *F. pratensis* would more correctly be included in the same genus than would *F. gigantea* and *F. arundinacea*. Even in the diploid-hexaploid hybrid (*L. perenne* \times *F. arundinacea*), it appears that a high proportion of the seven *Lolium* chromosomes find partners amongst the *Festuca* complement, yet in this interspecific hybrid seven chromosomes from each parent consistently fail to pair. The evidence shows that the true phylogenetic relationship of these plant groups is not indicated by their taxonomic position. Findings of this nature only emphasise the modern concept, that to procure the most useful classification of plants it is necessary to combine the knowledge

derived from cytological, genetical, ecological and taxonomic observations.

DISCUSSION.

Chromosome pairing and chiasma frequency.

Intergeneric hybrids in the Gramineae are not numerous. It is therefore especially interesting to consider the extent of chromosome pairing in some of the known cases. These are listed in Table V.

TABLE V.

Intergeneric hybrids	Metaphase associations				
	Uni-valents	Bi-valents	Tri-valents	Quadri-valents	Quinqui-valents
<i>Triticum vulgare</i> $n=21 \times$ <i>Secale cereale</i> $n=7$ (Thompson, 1926)	28	—	—	—	—
<i>Aegilops ovata</i> $n=14 \times$ <i>T. durum</i> $n=14$ (Sax, 1928; Bleier, 1928)	28	—	—	—	—
<i>Aeg. triuncialis</i> $n=14 \times$ <i>T. durum</i> $n=14$	Remainder	0-7	—	—	—
<i>Aeg. triuncialis</i> $n=14 \times$ <i>T. polonicum</i> $n=14$ (Kihara, 1929)	"	0-7	—	—	—
<i>T. vulgare</i> $n=21 \times$ <i>Aeg. cylindrica</i> $n=14$ (Kagawa, 1928)	21	7	—	—	—
<i>Zeamays</i> $n=10 \times$ <i>Texas Tripsacum</i> $n=36$ (Mangelsdorf and Reeves, 1931)	10	18	—	—	—
<i>Zea mays</i> $n=10 \times$ <i>Euchlaena perennis</i> $n=20$ (Longley, 1924)	6.7	7.3	6.7	—	—
Annual teosinte $n=10 \times$ <i>Zea mays</i> $n=10$ (Longley, 1924)	—	10	(F ₂ generation only)		
<i>L. perenne</i> $n=7 \times$ <i>F. pratensis</i> $n=7$	—	7	—	—	—
<i>F. pratensis</i> $n=7 \times$ (F ₁ of <i>L. perenne</i> \times <i>L. perenne</i> var. <i>multiflorum</i> $n=7$)	—	7	—	—	—
<i>L. perenne</i> $n=7 \times$ <i>F. arundinacea</i> $n=21$	9.7	8.3	0.4	0.4	0.1
<i>F. gigantea</i> $n=21 \times$ <i>F. arundinacea</i> $n=21$ or 22 (Present study)	14	Remainder	—	—	—

The above list is incomplete for *Aegilops-Triticum* hybrids, but there are no known cases where the number of bivalents exceeds seven.

The diploid *Festuca-Lolium* hybrids are unique in that there is perfect pairing between the parental chromosomes in the F₁. Since the F₂ of the annual teosinte-maize hybrid had regular pairing it would be expected that the F₁ would also be regular, but unfortunately that generation was not examined cytologically. Mangelsdorf and Reeves (1931) believe that the eighteen bivalents in the *Zea mays*-*Texas Tripsacum* cross represent pairing of the thirty-six *tripsacum* chromosomes. It has also been proved in this study that there is a certain amount of autosyndesis between the *F. arundinacea* chromosomes in the hybrid *L. perenne* \times *F. arundinacea*, as well as between chromosomes of one or both of the parental complements in the hybrid *F. gigantea* \times *F. arundinacea*. When autosyndesis occurs in unknown quantities it is obviously

impossible to make accurate deductions as to the relationships of the parental sets involved in the cross. It is, however, apparent from Table V that the pairing between the chromosomes of *Festuca* and *Lolium* is much more complete than that found in the other intergeneric hybrids with the possible exception of annual teosinte \times *Zea mays*.

It is universally agreed that pairing between chromosomes indicates that genetically they are at least partially homologous. There are several facts that must be kept in mind in making deductions of this kind. At one extreme there is complete pairing between the chromosome sets of species belonging to different genera such as *L. perenne* \times *F. pratensis* which are widely separated from each other taxonomically. At the other extreme there are abnormalities of chromosome pairing found in intra-specific hybrids between plants which appear to be closely related as indicated by genetical and taxonomic characters. One of the best examples of the latter is that of the five different chromosomal types found in the intraspecific hybrids of *Datura Stramonium* (Bergner and Blakeslee, 1930). Three of these types had different rings of four chromosomes, one has a configuration of eight and the other has twelve bivalents. Segmental interchange is most probably responsible for the differences between these types, but this does not necessarily involve any change in the genetic constitution.

Structural changes such as segmental interchange, inversion, translocation and reduplication have evidently not played any considerable part in the differentiation of *L. perenne* and *F. pratensis*; or if they have occurred they have not been perpetuated to any extent. These types of changes are, however, not unknown in this material, since one case is reported of a multiple association in a back-cross (17-bI-4) which can only be explained by structural hybridity.

Structural hybridity is relatively infrequent in the Gramineae. The only other cases known are found in *Anthoxanthum odoratum* var. *typicum* and *Briza media* (Katterman, 1931), *Zea mays* (Burnham, 1930) (McClintock, 1931) and Vilmorin's dwarf wheat (Huskins, 1931).

On the chiasma theory of pairing as developed by Darlington (1930*b*), it is recognised that numerous gene mutations could have occurred without the chromosome pairing being affected. However, large numbers of gene changes should lower the length of the chromosomes paired at zygotene, and this in turn would be expected to lower the chiasma frequency. The chiasma frequency of the F_1 of *L. perenne* \times *F. pratensis* was not significantly lower than either of the parents. It can be concluded therefore that the gene differences of these species are not

sufficiently frequent to be detectable by comparison of the chiasma frequencies of the parents with the F_1 . Although the effect of gene changes on prophase pairing has not been definitely established, the absence of any structural changes indicates that the former must have been mainly responsible for the genetic differences found in these two species.

In four of the derivatives of *Festuca-Lolium* hybrids (48-bE-1, 53-bE-1, 56-bE-3/2, 69-bI-1) the chiasma frequency was approximately half of that found in their sister plants or similar back-crosses. It is hardly conceivable that hybridity can be responsible for such marked differences in the sister plants, since meiosis in the immediate parents was normal, and structural changes of sufficient magnitude could not have occurred in individual gametes or during the course of a single sporophytic generation. Differences in the external environment are equally improbable as casual factors. It is concluded therefore that there is a segregation of genetical factors limiting chiasma formation. There are several comparable cases reported in the literature. Beadle (1930) found a recessive gene in maize which is responsible for asynapsis. The differences in chiasma frequency in different clones of *Fritillaria imperialis* is believed to be due to genetic variation (Darlington, 1930 b). In *Drosophila* a single factor has been found which causes practically complete linkage or lack of separation of any of the factors in any chromosome in the female (Gowen, 1928). Since the discovery of these genetic factors which are capable of modifying chiasma frequency and chromosome pairing in the whole complement, it is necessary to exercise additional caution in the interpretation of meiotic irregularities.

It might be expected that the genetic factors limiting chiasma frequency which have been found in the progeny of *Festuca-Lolium* hybrids could occasionally be detected in one or the other of the parents. Unfortunately the cytology of only a very few plants of the parental species has been studied. It has been found, however, that male sterile plants are frequent in *L. perenne* (Jenkin, 1931 c), and it is possible that some of these may exhibit a failure of pairing. On the other hand it is equally possible that these factors will only be effective in these intergeneric hybrids, and never in the parental species. Hollingshead (1930) found an example of this in interspecific hybrids of *Crepis capillaris* and *C. tectorum*. The *tectorum* parent was heterozygous for a lethal factor which halted the development of the hybrid to which it was transmitted, at the cotyledon stage. This lethal factor was ineffective in the parental species.

Fragmentation.

Both of the F_1 plants of the hybrid of *L. perenne* \times *F. arundinacea* and three of the back-cross plants to the male parent had more than the expected number of chromosomes. These supernumerary chromosomes probably arose by fragmentation, since they were about half the size of any of the other chromosomes. Numerous workers have observed fragmentation, and it has been shown to be induced by hybridity, X-rays, radium and "de nova" or by indeterminate environmental causes. It may either arise during meiosis or mitosis. A good example of the former was given by Darlington (1929 b) in *Tradescantia*. New fragments which had originated at the prophase of meiosis were observed in the pollen grain divisions. One of the earliest and most conclusive cases of fragmentation occurring in mitosis was reported by Navashin (1926). He observed that the *D*-chromosome of *Crepis tectorum* had fragmented, and that an attachment constriction had arisen "de nova" in the fragment in which one was lacking. In 1931 this author studied fragmentation and fusion induced by X-rays, and emphasised the importance of the attachment (kinetic) constriction for the survival of any chromosome, finding that the fragments usually fused with chromosomes which had attachment constrictions.

The supernumerary chromosomes found in this study have evidently arisen early in the sporophytic generation, since they were regularly found in the root tips of the F_1 . Furthermore these fragments had evidently developed a new attachment constriction and were capable of survival through both mitotic and meiotic divisions, since they could be observed in some of the plants of the succeeding generation. It is suggested that fragmentation took place in one of the arms of the longer V-shaped chromosomes, and if this had occurred the portion that retained the old attachment constriction would be sufficiently long to be indistinguishable from the shortest of the unfragmented chromosomes.

Why fragmentation is induced in the hybrids of *L. perenne* \times *F. arundinacea* and in the back-crosses to the male parent is unknown. Additional observations on large numbers of *F. arundinacea* plants may possibly reveal that the phenomenon is frequent in this species, and not especially limited to its progeny when crossed with *L. perenne*.

Morphological and physiological abnormalities.

Structural and functional abnormalities were observed in certain of the *Festuca-Lolium* hybrids at various stages during the development of

the floral structure and during meiosis. It is seldom that any marked morphological and physiological abnormalities are apparent in the foliage. One exception to this was reported by Jenkin (1924, 1930) in the F_1 of *F. rubra* \times *F. arundinacea*. The seedlings were very weak, the resulting plants developed slowly, and while it was just possible to keep them alive they failed entirely to produce any inflorescences. In the present study the earliest and most remarkable irregularity of flower structure was observed in one of the plants (101-bE-1) of the back-cross to the male parent of a hybrid derivative of *L. perenne* \times *F. arundinacea*. In this plant supernumerary ovaries or bract-like structures replace the anthers. The unusual genetical constitution of this particular plant had evidently modified the normal differentiation of the male sex organs, so that in some of the florets, development in the direction of femaleness replaced anther development. In other florets there seemed to be a complete absence of differentiation towards either sex in the anther primordia, so that bract- or leaf-like structures were developed in the position where the anthers were normally formed. This latter point may have evolutionary significance regarding the origin of the anthers from modified leaves. Somewhat comparable cases were found in *Hyacinthus orientalis* by Nemec (1898) (quoted by Schürhoff, 1926) and Stow (1930). Embryo sac-like giant pollen grains were produced in the anthers. The abnormalities observed by Stow were induced by subjecting the bulbs to a high temperature at the stage when the reduction division was taking place.

The first evidences of degeneration, apart from the floral abnormalities noted above, was the failure of wall formation commencing in the last few somatic divisions of the sporogenous tissue and occurring at various stages throughout meiosis. Giant multinucleate pollen mother cells were produced. Gaines and Aase (1926) found very similar abnormalities in a haploid plant which was supposed to have arisen from *Triticum compactum*. These multinucleate structures often give the false impression of cell fusion. Kagawa (1928) illustrates a fusion of pollen mother cells in the F_1 of a hybrid between *T. vulgare* and *Aegilops cylindrica*. It is suspected that this is also the result of failure of wall formation rather than a fusion in the strictest sense. Moffett (1932) found numerous diploid and tetraploid pollen grains in diploid *Kniphofia*. He concluded that it was due to failure of wall formation which in turn was the result of failure of the spindle mechanism.

Binucleate pollen mother cells were observed in plant 44-bI-1. These frequently resulted in syndiploid metaphase plates when the chromosomes became orientated on a common spindle. Darlington (1930 a) made

similar observations in *Prunus avium*. The following are some of the researches in which binucleate pollen mother cells have been observed: Gates and Rees (1921) in *Lactuca sativa*; Karpechenko (1927) in hybrids of *Raphanus sativa* \times *Brassica oleracea*; Fukushima (1931) in *Brassica japonica*.

In those hybrid plants which produce only a low percentage of good pollen or none at all there are usually definite signs of general degeneration following tetrad formation. The tapetal layer, in addition to most of the pollen, rapidly takes on an unhealthy appearance. This may or may not be the result of the irregularities which arose in consequence of failure of pairing. Any of this pollen, which happened to receive a genetically balanced complement of chromosomes, would have little chance of survival. In contrast to this a number of hybrid plants were found which produced over 50 per cent. good pollen. Some of these had unpaired chromosomes and the usual meiotic irregularities though there did not appear to be any general degeneration as above. But certain of the pollen grains appeared to be unhealthy, and these are believed to be the ones which had received a genetically unbalanced complement.

In general it can be said of all the hybrids in this investigation that it is only the minority in which male sterility was the direct result of failure of pairing and its consequent meiotic irregularities. The remainder of the plants are physiologically incapable of producing viable pollen. The abnormal physiological condition may be expressed in (1) a failure of the spindle and wall formation, (2) a precocious degeneration of tapetum and the microspores usually following tetrad formation.

SUMMARY.

1. Evidence on the genetical relationship of the parents of the diploid hybrids between *Lolium* and *Festuca* was determined from calculations of the chiasma frequencies at the heterotypic metaphase in the F_1 .

2. A segregation of genetical factors limiting chiasma frequency was observed in the back-crosses.

3. The presence of chains of four chromosomes in the heterotypic metaphase in one of the diploid hybrids indicated that translocation, reduplication or interchange had occurred between non-homologous chromosomes in one of the parental species.

4. In the diploid-hexaploid cross (*L. perenne* \times *F. arundinacea*) a high proportion of the *Lolium* chromosomes paired with their homologues in the *Festuca* complement, and there was also a small amount of auto-syndesis among the chromosomes of the latter.

5. Fragmentation was found to occur in the F_1 of *L. perenne* \times *F. arundinacea* and in every back-cross to the male parent where it was possible to be reasonably certain of the constitution of the parental gametes.

6. A demonstration of genetical crossing-over was afforded by the occurrence of an unusual quadrivalent association in the heterotypic metaphase of one of the hybrid derivatives.

7. An attempt was made to determine the correlation between chromosome number, meiotic behaviour and the percentage of good pollen in a group of hybrid plants with a series of chromosome numbers ranging from 14 to 23.

8. Deductions as to the immediate cause of male sterility were made from the appearance of the various floral and meiotic abnormalities in the hybrid plants.

9. The probable origin of the supposed natural hybrids (*Festuca loliacea*) was considered.

10. In the F_1 of a cross between *F. gigantea* and *F. arundinacea*, seven chromosomes from each parent remained unpaired. The cytological evidence of hybridity was more pronounced in this interspecific hybrid than in the intergeneric hybrids between *Lolium perenne* and *Festuca pratensis*.

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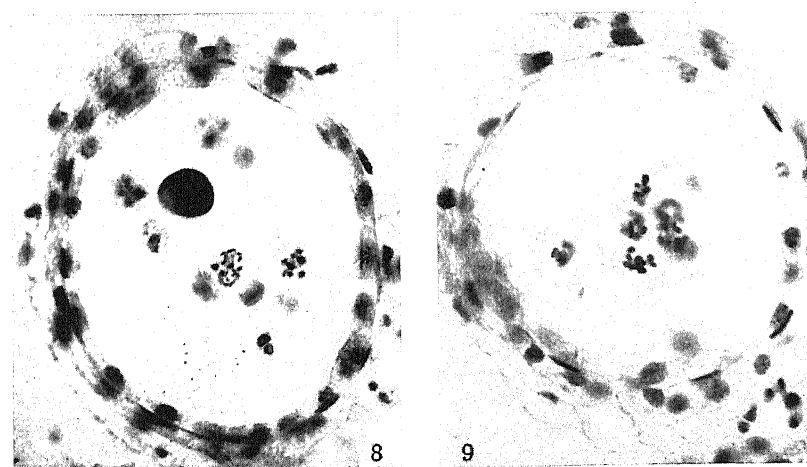
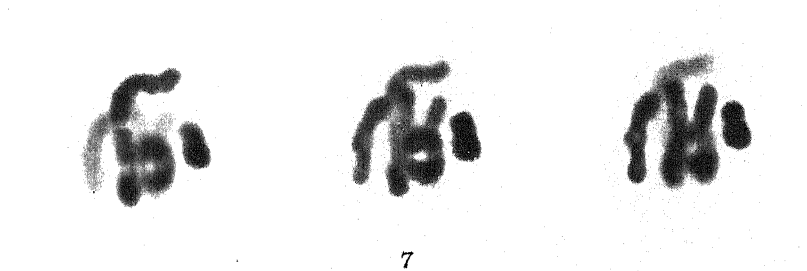
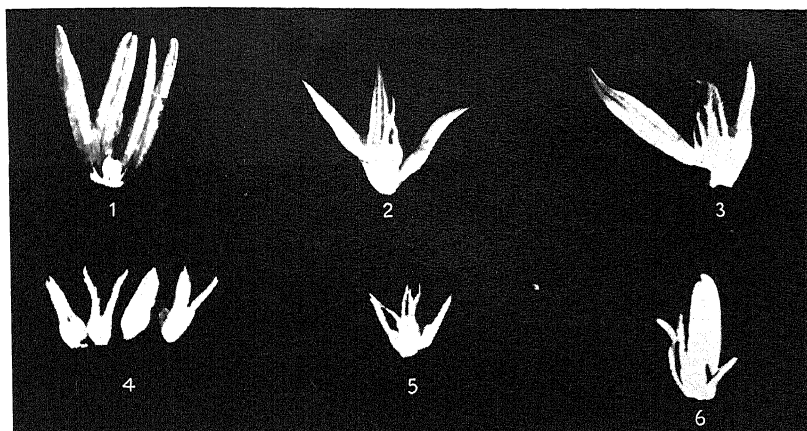
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EXPLANATION OF PLATE VII.

- Fig. 1. Normal sexual organs in 101-bE-4.
- Figs. 2-5. Abnormal sexual organs in 101-bE-1.
- Fig. 2. Three bracts replace the anthers.
- Fig. 3. One bract, one deformed anther and one small pistil replace the anthers.
- Fig. 4. Three apparently normal pistils replace the anthers.
- Fig. 5. Three apparently normal pistils replace the anthers.
- Fig. 6. Abnormal anthers in 49-bI-1.
- Fig. 7. Heterotypic metaphase of 17-bI-4; note chain of four chromosomes. The whole complement is shown by photographs at three different foci. × 3100.
- Fig. 8. Locule of anther in 44-bI-1 containing cells at various meiotic stages. × 300.
- Fig. 9. Locule of anther in 44-bI-1 containing multinucleate pollen mother cell. × 300.



THE CHROMOSOMAL CONSTITUTION OF CERTAIN CULTIVATED APPLE VARIETIES.

By MURIEL V. ROSCOE.

(With Eight Text-figures.)

INTRODUCTION.

THE cytology of many varieties of the cultivated apple has already been studied by several workers including Rybin (1926, 1927), Shoemaker (1926), Kobel (1926*a*, 1926*b*, 1931), Heilborn (1928), Nebel (1929, 1930*a*, 1930*b*), Darlington and Moffett (1930) and Moffett (1931). Through these studies there has developed an interest in the relationship of chromosomal constitution to some practical problems of the grower and breeder. Some of these aspects have been discussed by Crane and Lawrence (1929, 1930), and of particular interest is their treatment of polyploidy and its bearing on fertility and on seedling vigour. Correlations between diploidy and triploidy and pollination may be made from the pollen-germination experiments of Florin (1926) and Kobel (1931).

Most recent among the investigations on apples are those of Brittain and Eidt, who have been working with pollination and genetical aspects of diploid *v.* triploid varieties. These experiments have been carried on chiefly at the Dominion Experimental Station at Kentville, a station located in the Annapolis-Cornwallis Valley, the apple-raising section of Nova Scotia. Their data show correlations between ploidy and effective pollination, set of fruit, seed count, seed germination and growth rate of seedlings.

The present cytological work was carried on during the summer and fall of 1932 and developed out of an interest in the experiments of Brittain and Eidt. Some eighteen varieties were chosen for study and included in these were certain varieties extensively grown in the Annapolis Valley region¹. Among such are Wagner, Golden Russet, Nonpareil (Roxbury Russet), Stark and Fallawater.

MATERIALS AND METHODS.

The material for the study was obtained from the orchards of the Dominion Experimental Station at Kentville, with auxiliary material

¹ The pollination and genetical aspects of these varieties as ♂ and ♀ parents are presented by Brittain and Eidt.

from cuttings in the case of Golden Russet, Nonpareil and Stark. The cuttings were developed in the greenhouse and the pollen mother cell divisions from these gave appearances very like those of buds developing out of doors.

Carnoy's fluid was used as a fixing agent and the buds embedded in celloidin. The sections were stained with Heidenhain's iron-alum haematoxylin and studied with the aid of 1.5 mm. and 2 mm. Zeiss apochromatic objectives used with 10× and 15× oculars.

Observations have been concerned chiefly with the reduction divisions of the pollen mother cell, but in several instances these have been accompanied by study of somatic divisions. Petal, stamen and ovary tissue afforded good opportunities for making somatic counts.

OBSERVATIONS.

Banks' Crimson Gravenstein. $2n = 51$ (somatic and reduction divisions).

The triploid condition of this variety places it with the clonal varieties of Gravenstein reported by Nebel (1930 *b*). Petal tissue has provided somatic divisions showing 51 chromosomes. Reduction divisions reveal M. I plates with 22 and 23 units comparable with those in Stark. Irregular chromosomal distribution in both the heterotypic and homotypic divisions followed by polyspory indicates the similarity of division phenomena in this and varieties such as Stark, Nonpareil and Fallawater.

Crimson Beauty of New Brunswick (Early Red Bird). $2n = 34$; $n = 17$ (M. I).

The reduction divisions are featured by regularity with normal interkinesis and tetracary.

Deacon Jones. $2n = ca. 34$ (tapetal cells); $n = 17$ (M. I and A. I).

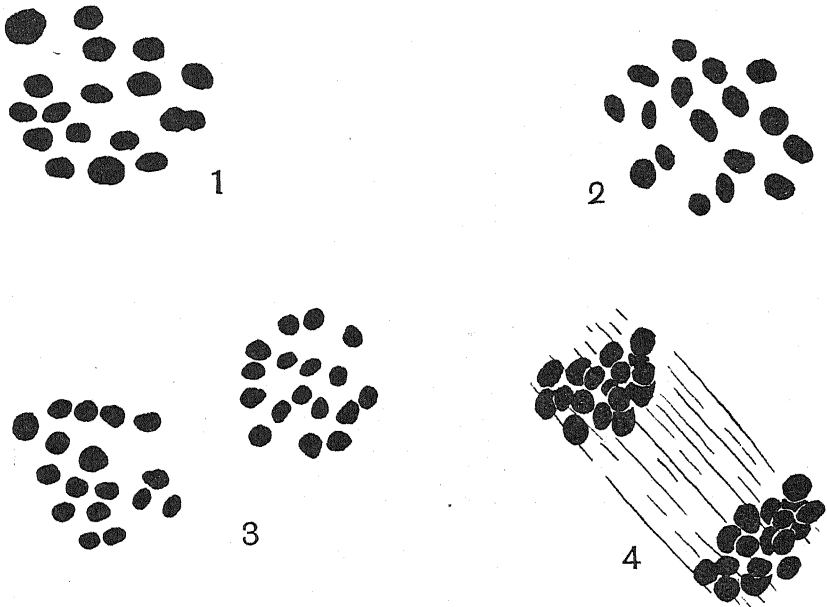
Most M. I divisions show 17 bivalent chromosomes at the plate, but in a few instances there are 16 and 15. This means that probably one and two of these were quadrivalents, although such a morphological nature was not recognisable. With this condition occurring even rarely, it is considered possible that the form is similar to the diploid species with complex chromosomes examined by Darlington and Moffett. The rarity of such occurrences in my material should be stressed. The divisions are regular and only a few A. I cases show slight lagging. All the chromosomes are eventually included in the interkinetic nuclei.

Delicious. $n = 17$ (M. I, M. II, A. II).

The regular divisions lead to normal interkinesis and tetracary. Shoemaker has reported Delicious as having $n = 14$ chromosomes, but it is apparent from the counts in both heterotypic and homotypic divisions that the number is 17 and that the behaviour is that of an ordinary diploid. Fig. 3 shows 17 chromosomes in each of the metaphase plates of the homotypic division.

Duchess. $n = 17$ (M. I, A. I.)

Nebel lists this as a diploid form, Charlamowsky (Duchess of) Oldenburg. The count and the chromosomal action confirm Nebel's report.



Figs. 1-4. Diploid varieties. 1, Golden Russet: M. I, polar view, showing 17 chromosomes. 2, Wellington: M. I, polar view showing 17 chromosomes. 3, Delicious: M. II, 17 chromosomes in each plate. 4, Wolf River: A. I featured by regularity of chromosomal behaviour.

Fallawater. $2n = 51$ (somatic divisions).

Somatic metaphase plates observed in cells of petal tissue indicate a triploid condition for this variety. Only a limited number of pollen mother cells were available for a study of reduction divisions. M. I plates observed showed 21 chromosomal units. The conspicuous lagging of chromosomes in heterotypic figures indicates a similarity of behaviour for Fallawater, Stark and Nonpareil.

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Golden Russet. $n = 17$ (M. I, M. II, A. II).

Fig. 1 is a drawing of a polar view of an M. I plate, and emphasises the count of 17. The divisions are typical of diploid forms and do not show lagging or irregularity of action.

Grimes Golden. $n = 17$ (M. I, T. I).

The variety has regular divisions.

Jonathan. $n = 17$ (M. II).

While no phases of the heterotypic division have been seen for this variety, the regularity of the interkinetic figures, coupled with the constancy of 17 in the M. II plates, leaves no doubt as to its diploid character.

Nonpareil (Roxbury Russet). $2n = 51$ (reduction divisions).

The difficulty of making counts for triploid forms is especially great in working with reduction divisions only. As Darlington and Moffett point out, the tendency toward multiple association reaches greater limits in triploid than in diploid species. However, the units which have been seen in Nonpareil do not range higher than quadrivalents and in this respect differ from the triploids reported upon by these English workers. Bivalents are represented in greater numbers than the quadrivalents, trivalents or univalents. Diakinesis reveals varying counts, but always in excess of 19. M. I exhibits units ranging from 20 to 29. Fig. 6 illustrates a plate with 29 such units. A determination of the number of quadrivalents, trivalents, bivalents and univalents in this and other cases shows them to aggregate 51. Diakinetic figures are thus supported by M. I and A. I findings. Interkinesis shows chromosomes unincorporated in the nuclei, and the homotypic divisions very often result in polyspory.

The lagging of the chromosomes in the first division is pronounced and late anaphases show univalents scattered all along the spindle. Lateral views all show the tardy action of the chromosomes. M. II plates show 24 to 25 chromosomes in one plate with many fewer in the second plate. A. II may reveal a group entirely free from the spindle and lying in the cytoplasm.

While the larger number of observations were made on greenhouse material, these were confirmed by the figures from buds collected out of doors.

Red Spy. $n = 17$ (M. I).

Lateral views of M. I, T. I and interkinesis show regular chromosomal distribution which leads to the formation of orthoploid tetraspores.

Reinette Rouge d'Hiver. $n = 17$ (M. I, T. I, M. II).

Lateral views of M. I, T. I and T. II exhibit meiotic regularity.

Stark. $2n = 51$ (reduction divisions).

In M. I of this variety 22-25 chromosomes are usually present and these are represented by varying numbers of quadrivalents, trivalents, bivalents and univalents. Fig. 5 shows a metaphase plate with 23 chromosomes.

The table below suggests the variability of the chromosomal association observed at metaphase, but at the same time emphasises the triploid nature of the variety.

Quadri- valents	Trivalents	Bivalents	Univalents	Units	Total
5	3	8	6	22	51
1	7	12	2	22	51
1	5	13	6	25	51
0	7	14	2	23	51

It is seen that the total count is always 51 in these M. I plates and the triploid character is vouched for by the M. II figures. M. II plates differ greatly in counts, but cells showing plates with 28 and 23 chromosomes (Fig. 8) or with plates of 28 and 19 chromosomes and 4 separate chromosomes forming a third small plate on a separate spindle confirm the heterotypic counts. All figures of both divisions are distinguished by lagging of the chromosomes. Fig. 7 is a drawing of an anaphase illustrating such lagging. Most homotypic divisions show chromosomes lying off the spindles and frequently also supernumerary spindles are present. Polycary, polyspory and even diad formations result. Such figures are similar to those described by Rybin in the triploid *Reinette du Canada*.

Wagner. $n = 17$ (M. I, M. II, A. II, T. II).

M. I shows 17 bivalent chromosomes constantly and the regularity of the division stages in the form is impressive.

Wellington. $2n = ca. 34$; $n = 17$ (M. I, A. I, T. I, M. II).

Lateral views of M. I, A. I, and T. I all show great regularity.

Winter Banana. $2n = 34$; $n = 17$ (diakinesis and M. I).

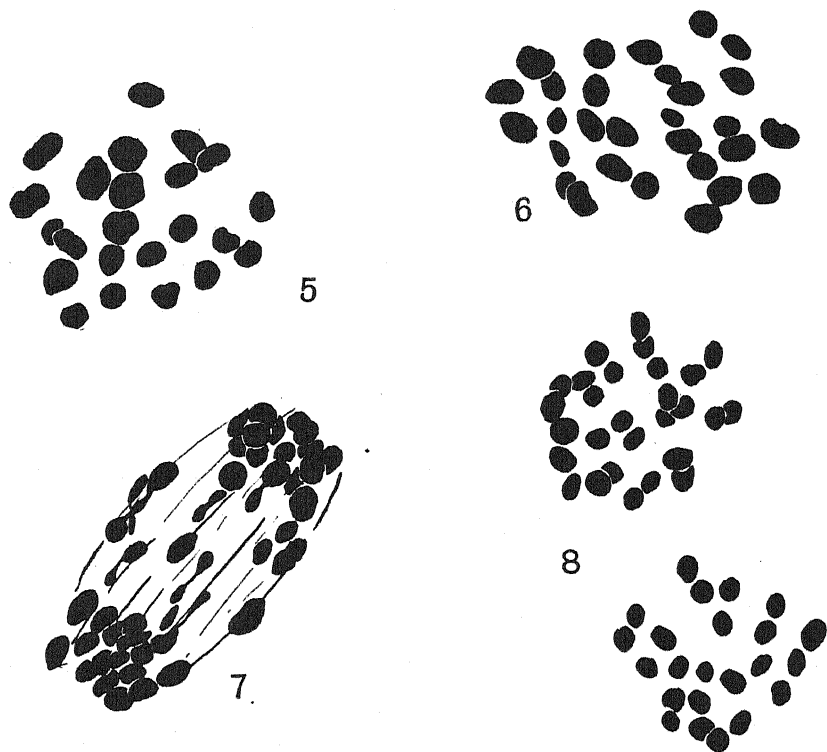
Division figures show complete absence of lagging chromosomes. Chance observation of a somatic division in a tapetal cell gave 68 chromo-

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somes as the total for the metaphase plates of the two nuclei, leading to a confirmation of the reduced number, 17, in the pollen mother cell.

Wolf River. $n = 17$ (M. I and M. II).

The regularity of division figures is marked.



Figs. 5-8. Triploid varieties. 5, Stark: M. I plate with 23 chromosomes (6 trivalents, 16 bivalents, 1 univalent). 6, Nonpareil (Roxbury Russet): M. I plate with 29 chromosomes. 7, Stark: A. I, featured by irregularity of chromosomal behaviour. 8, Stark: M. II plates of 28 and 23 chromosomes show inequality of distribution in the heterotypic division.

York Imperial. $2n = 34$; $n = 17$ (M. I, M. II).

The heterotypic divisions show more lethargy on the part of the chromosomes than in the preceding diploids and in some cells exhibit slight lagging. The results are not serious since, as shown by the M. II counts, all the chromosomes are included in the interkinetic nuclei. The homotypic division is regular in the distribution of the chromosomes.

DISCUSSION.

Diploidy, triploidy and aneuploidy.

With 34 and 51 as somatic counts the varieties of this investigation are all shown to be euploid. Since Rybin's publication of Reinette du Canada as a triploid in 1927, many varieties have been admitted to this category. The re-examination by Nebel and by Darlington and Moffett of some of Kobel's aneuploid varieties has shown these to be triploid¹. The revised counts have been accepted by Kobel and indeed supported by his recent findings in Bohnapfel (1931). Nebel (1929) noted the strong tendency toward euploidy in apples and the data now at hand cover a large number of varieties and strongly support his earlier conclusion.

Darlington and Moffett along with Crane and Lawrence find that aneuploid seedlings which were derived from triploid parents (selfed or crossed with diploids) are not vigorous, and from the work already done, it seems safe to predict that rarely will aneuploid forms appear in the cultivated apple.

Kobel earlier (1926 *b*) thought that the poor germination of pollen in certain varieties of apples and pears was due to the presence of abnormal chromosome numbers and supported this for the apple by his counts for Warner's King, Bohnapfel, Gravenstein and Schöner von Boskoop. In view of the revised counts for Gravenstein and Schöner von Boskoop referred to above, it is suggested that triploidy and not aneuploidy is associated with low germination percentages.

Chromosomal behaviour.

A study of the species described reveals that the meiotic divisions of the triploid species vary considerably from those of the diploid species. In diploids, the regularity with which the chromosomes separate and pass to the poles in the heterotypic division is in most cases pronounced. This regularity is maintained in the homotypic division. Such a normal course of behaviour has been mentioned by Darlington and Moffett for the diploid varieties of their investigation. Of these they say: "Abnormalities—such as the occasional lagging of univalents—are of sporadic occurrence." Heilborn studied divisions in buds of diploids which had been developed in a warm greenhouse and believed the irregularities had been largely induced by increased temperatures. He thus attributed the cause of irregular meiosis to outside factors. Nebel found that

¹ The varieties examined include Ribston Pippin, Gravenstein, Baldwin and Belle de Boskoop (Nebel, 1929) and Bramley's Seedling and Crimson Bramley (Darlington and Moffett, 1930).

material of a given variety showed greater irregularities in his 1927 than in his 1928 collections, and in some diploids quite an amount of irregularity is recorded (*Malus baccata*, *M. scheideckeri*, *Malus* var. Yellow Newtown). Nebel thinks the explanation is to be found in unfavourable environmental conditions rather than in any inherent factors. The findings of the present investigation are such as to indicate a pronounced regularity of division for most diploids. Any irregularity of meiosis is so slight that the effect upon spore formation may be considered negligible.

For triploid varieties, chromosomal behaviour is, as may be noted in foregoing descriptions of Stark and Nonpareil, quite different from that in the diploid species. Varying numbers of chromosomes are directed toward different poles, and varying numbers of laggards are seen on the spindle. This means in most cases that the nuclei of interkinesis contain different numbers of chromosomes, while frequently some of the laggards are not included in these daughter nuclei. Homotypic divisions reveal still further instances of lagging chromosomes and abnormal figures which lead to the conditions of polycary and polyspory. The effect upon the formation and functional ability of the pollen is obviously deleterious. It is apparent from the foregoing account of meiosis that distribution of chromosomes in triploids is such as to lead to morphologically and physiologically poor pollen.

It is to be noted that the present investigation deals with diploid and triploid varieties subjected to the same environmental influences. The contrast in chromosomal action in the two cases is significant.

Causal factors in meiosis.

The study of chromosomal behaviour in triploids has already received much attention, and the meiotic phenomena in such are now well known. Although triploid species and varieties doubtless originate in different ways, it is generally conceded that hybridisation is a frequent cause.

Woodworth (1929) has described meiosis for *Betula jackii*, undoubtedly a hybrid between *B. lenta* (diploid) and *B. pumila* (tetraploid). Of the meiotic irregularities he says: "The apparent cause...is the lack of homology between the chromosomes during gemini formation." Here the irregularities are attributed to the hybrid ancestry, and are concerned not only with numerical constitution but also with the non-homology of the chromosomes entering into that constitution.

While the origin of triploid varieties in the apple is not necessarily analogous with that of the triploid species of *Betula* referred to, the

meiotic behaviour of the triploid apple varieties under consideration is of the nature of meiotic behaviour in triploids with a hybrid origin.

For the triploid apple the non-homology of the units is indicated by the great variety of their associations as represented by the formation of quadrivalents, trivalents, etc. Darlington and Moffett have pointed out that various sorts of multivalent associations occur in *Malus* varieties, and that meiotic irregularities lead to dissimilar numbers in the homotypic plates. Nebel found two modes of conjugation in *M. spectabilis*, with the forming of 17 trivalent groups in the one case, and of 25 bivalent groups in the other case. The present work gives further instances of such associations for triploid varieties of the cultivated apple. Table I shows that for Stark various counts between 22 and 25 may occur. A greater range of counts was found for Nonpareil and although the majority of these were between 22 and 25, yet variable numbers ranging as high as 29 (Fig. 6) have been found. Apparently, in the case of Stark and perhaps for the majority of the cases in Nonpareil, the "third supposed haploid set," to borrow Darlington and Moffett's term, pair among themselves. In the remaining cases in Nonpareil, where more than 25 units are present, it seems that there has not been complete pairing within the "third haploid set."

Darlington and Moffett state that the meiotic abnormalities in triploids lie in the "multivalent association of chromosomes." If this in itself were an explanation of the *cause* of the irregularities observed in triploids, the same logic would lead us to expect more prominent irregularities in the reduction divisions in diploids, since in the diploid species which these workers have described quadrivalent and sexivalent chromosomes are commonly present.

The balanced or unbalanced relationship seems to bear on meiotic behaviour, and from the results of the present investigation, it is believed that for diploids the association of homologous units with a balance of their somatic number, $2n = 34$, accounts for the general normality of the division figures. Similarly, it is believed that the triploids show the irregularities manifest in triploids of hybrid origin, and that the unbalanced number and the non-homology of the units concerned can be considered the causative agencies of such abnormalities.

SUMMARY.

1. Of the eighteen varieties of cultivated apples studied, fourteen have been found to be diploid and four triploid.
2. Euploidy is characteristic of cultivated apples.

3. The diploid varieties considered are distinguished by regularity of meiosis with normal chromosome distribution.

4. The triploid varieties are distinguished by irregularity of meiosis with unequal distribution of the chromosomes.

5. Certain similarities between the division figures of triploid varieties and hybrid forms are pointed out.

6. It is considered that constitutional rather than environmental factors determine chromosomal behaviour.

7. Homology and a balanced relationship of the chromosomes account for the normal reduction figures in diploid varieties.

I am indebted to Dr W. H. Brittain of Macdonald College for assistance rendered during the course of the investigation. I wish also to thank Mr C. C. Eidt of the Dominion Experimental Station at Kentville for his kind co-operation and especially for his services in securing material from the Experimental Station orchards.

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THE GENETICS OF *PAPAVER COMMUTATUM* AND ITS HYBRIDS WITH *PAPAVER RHOEAS*.

BY JAMES PHILP.

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PAPAVER COMMUTATUM Fisch. and Mey. has 14 somatic chromosomes (like *P. Rhoeas* Linn.). The petals are crimson with a large black blotch near the middle of the petal, the filaments and anthers are black and the pollen is olive green. The capsule is globular with narrow dark stigmatic rays, and hairs at the top of the flower-stalk are generally strigose but may be patent. The latex is white.

A form occurs having creamy white petals with a yellowish ghost of a blotch, white filaments, yellow pollen, white stigmatic rays and almost colourless latex. This form in these experiments is often less vigorous than the crimson form.

The group of characters, crimson petals, black blotch, filaments, anthers and stigmatic rays, and white latex behave genetically as if they are governed by a single factor which is completely dominant to the corresponding recessive characters of the white form **w**. Half of a petal of a heterozygote has been observed to mutate to the recessive white form.

Strigose hairs are incompletely dominant to patent hairs **p**, and these characters are controlled by a single factor difference. The factors **w** and **p** are independent.

The observed results given in Table I are in close agreement with expectation based on the above interpretation. In the two families from

TABLE I.

Cross	Number of families		++	+p	w+	wp
++++ × ww+p	1		41	0	0	0
		Expectation	41.0	0	0	0
+w+p × +w+p	2		125	51	65	19
		Expectation	146.25	48.75	48.75	16.25
+w+p × ww+p	1		17	8	15	6
		Expectation	17.25	5.75	17.25	5.75
ww+p × ww+p	2		0	0	5	2
		Expectation	0	0	5.25	1.75

the cross **ww+p** × **ww+p** thirty-three seedlings were obtained although only seven reached maturity; twenty-five were green and eight

were albinos. This ratio is very close to expectation, 24.75 : 8.25, on the assumption that the recessive albinism differs from the normal green by a single factor.

Apart from the above variation the other characters, such as foliage, habit and hair colour, were remarkably constant. The hairs were white on the stem and bud, but those near the base of the bud were brownish yellow.

HYBRIDS BETWEEN *P. RHOEAS* AND *P. COMMUTATUM*.

Twenty-two hybrids were obtained from a cross between a plant of *P. Rhoeas* used as a female and a plant of *P. commutatum*. The *Rhoeas* parent had the typical scarlet flower colour and patent hairs, also red hairs on the stem and bud, anthocyanin in the stem and very pale yellow latex. The *commutatum* parent was a crimson-flowered form with strigose hairs.

The hybrids were rather weakly and only nine flowered. The flower colour and leaf shape were remarkably uniform throughout. The flower colour was very like that of *P. commutatum* but had a trace of the scarlet of *P. Rhoeas*. The black blotch was also similar to that of *P. commutatum* in shape, size and position (carried up nearer the centre of the petal and not confined to the base as in *P. Rhoeas*). Likewise the flower shape tended to be cup-shaped as in *P. commutatum*. The lower leaves in particular tended very much towards the shape of those of *P. commutatum*, but the upper leaves were more serrated. Only ten plants could be scored for latex colour and all had white latex.

Segregation occurred for colour and form of the hairs and for anthocyanin in the stem. Six plants had strigose hairs and ten had patent hairs—presumably a 1:1 ratio owing to the heterozygosity of the *commutatum* parent. Six plants had red hairs on the bud or stem or both and three had white hairs on both. Three others had white hairs on the stem, but the hairs could not be scored. Nine plants had anthocyanin in the stem and two lacked anthocyanin.

A cross was also made between a plant of a cultivated variety of *P. Rhoeas* as female and a plant of *P. commutatum* of the same type as in the previous cross. The female parent had mauve petals with a white blotch, white filaments, yellow pollen, patent hairs and deep yellow latex. This plant differed from the wild in four factors for flower colour **bpit** (Philp, 1933).

Fifteen hybrids were raised, and in this case they were quite vigorous. Again the floral characters and leaf shape were uniform and were very

similar to those of *commutatum*. All had very pale yellow latex (almost white). Seven had strigose hairs and eight had patent hairs.

Both families of hybrids were self-sterile and many of the flowers had poorly developed anthers. The hybrids with wild *P. Rhoeas* set no natural seed although grown in close proximity to forms of *Rhoeas*. Crosses were made between the second lot of hybrids as female and cultivated forms of *Rhoeas* and they were also unsuccessful.

The late Mr W. C. F. Newton made a cross between a Shirley poppy as female and *P. commutatum*. The Shirley parent had pale rose-pink petals, white blotch, white filaments, yellow anthers and patent hairs and presumably differed from wild *P. Rhoeas* in three factors for flower colour **cbt**.

Five hybrids had strigose hairs, two had patent hairs and all had a black blotch. Natural seed was obtained from one hybrid with strigose hairs, and Newton recorded that one of the progeny was a triploid with strigose hairs and the petal colour crimson with a large black blotch reaching to the base of the petal. Natural seedlings were obtained from this triploid, some of which had a white petal blotch and crosses were made between them. The author studied the progeny from these crosses and found that the plants were diploids of the *Rhoeas* type except that they segregated for strigose and patent hairs. The results showed that strigose hairs were dominant to patent hairs, and on the basis of a single factor difference there was generally a deficiency of strigose plants.

Since this work was carried out seed was kindly provided by Dr Heimburch of a strain of *P. commutatum* called Burbank's "Silver Lining." This form is peculiar in that the outer surface of the petals has the typical crimson colour and black blotch of *P. commutatum*, while the inner surface of the petals is creamy white with a ghost of a blotch as in the recessive form here mentioned. The margin of the inner surface of the petals is crimson with the white region forming striations into it. This form breeds true.

DISCUSSION.

Comparison of the known genetical constitution for flower colour in *P. commutatum* and *P. Rhoeas* shows considerable differences. The crimson colour and black blotch, filaments and anthers of *P. commutatum* and the recessive white form with white blotch and filaments and yellow anthers behave genetically as if they were controlled by a single factor difference or by a group of very closely linked factors.

The scarlet colour and black blotch, filaments and anthers of *P.*

Rhoeas on the other hand are governed by several factors, and in order to produce a creamy white flower comparable to that form of *P. commutatum* it would be necessary to have four factors, **btci**, in the recessive condition (Philp, 1933). **b** dilutes the petal colour and produces a white blotch and yellow anthers. **t** presumably causes the absence of a petal pigment, and together with **b** causes the filaments to be white. **c** further dilutes the petal colour, and along with **i**, which produces flavone, causes it to be creamy white. Both forms of *P. commutatum* contain flavone. **t** and **i** are closely linked and **b** and **c** are independent of one another and of **t** and **i**.

Obviously the expression of the factor **w** in *P. commutatum* is somewhat similar to that of **b** in *P. Rhoeas*, but it has a much greater influence. Further the allelomorph of **w** in the interspecific hybrid is largely epistatic over the allelomorph of **b**.

The habit and foliage characters of *P. commutatum* also are largely dominant over those of *P. Rhoeas*, and likewise the white latex appears to be dominant over the yellow latex forms of *P. Rhoeas*, although within *P. Rhoeas* white is recessive to yellow.

The evidence also suggests that the strigose hairs of *P. commutatum* are dominant to the patent hairs of *P. Rhoeas* and that the two characters are probably controlled by a pair of allelomorphs.

The fact that the two species hybridise indicates some relationship between them, and the above evidence substantiates this in that although there are some wide genetical differences, there are some factors which are probably common or similar in both species.

Cytological observations also show that the chromosomes of both species are homologous to a considerable extent but by no means completely. In the parental species seven bivalents are regularly formed at first metaphase in meiosis, whereas in the hybrid (with wild *P. Rhoeas* as one parent) a varying number of bivalents are formed, the remainder of the chromosomes remaining unpaired. The following observations were made on twenty-five pollen mother cells:

Number of bivalents	1	2	3	4	5	6	7
Number of cells	0	0	2	8	10	4	1

P. commutatum appears to be remarkably stable, the only variations being the three forms of flower colour, the two forms of hairs and some slight variation in habit and foliage—dwarf and narrow lobes.

Wild *P. Rhoeas* shows variation for the colour of the hairs and the latex and a remarkable range of variation of habit and foliage. *P. Rhoeas* var. *strigosum* has the same range of variation and geographic distribution as *P. Rhoeas* and is distinguished from *P. Rhoeas* by its having strigose

hairs. Presumably these two forms differ only in the hair form, and this is probably due to a single factor difference¹. In that case *P. commutatum* and *P. Rhoëas* show a similar variation in hair form.

To the author's knowledge only two variants in flower colour in the wild have been reported—*violaceum* (p) and rose (presumably b). This is rather surprising, since eight factors for flower colour are known in *P. Rhoëas* and its cultivated forms (Philp, 1933). Why is flower colour so constant in the wild as opposed to foliage and habit? It may be mentioned that plants differing from the wild type in all eight factors for flower colour have occurred in culture and were quite vigorous and fertile. This evidence suggests that these allelomorphs to the wild factors for flower colour are not associated with some form of lethal factor which would be selected out in nature.

The origin of most of these forms of flower colour which are in cultivation is not known, and it may be suspected that some of them have been introduced by hybridisation with other species. An example of how this could occur has already been given here with regard to the introduction of strigose hairs from *P. commutatum* into cultivated forms of *P. Rhoëas*. If the parentage of these plants which were apparently forms of *P. Rhoëas* had not been known it might have been considered that a dominant mutation had occurred giving rise to strigose hairs. Such hybridisation in the production of the cultivated forms of *P. Rhoëas* might account for the difference in vigour and fertility between the hybrids of wild and cultivated forms of *P. Rhoëas* with *P. commutatum*.

From the above considerations it may be concluded that *P. commutatum* is very homozygous, which is unusual in a naturally cross-fertilised plant. *P. Rhoëas*, on the other hand, is relatively very heterozygous. It follows therefore that natural selection has probably been much more intense in the case of *P. commutatum* than in *P. Rhoëas*, and this might be the cause or the result of the more limited geographic distribution of *P. commutatum*. Actually *P. commutatum* is mainly confined to southern Russia, Asia Minor, Persia, the Caucasus, Armenia and the Isle of Thasos, while *P. Rhoëas* is found practically over the whole of Europe, in the Near East, and in North Africa.

The homozygosity of *P. commutatum* may also explain its general

¹ Since this paper went to Press, the author has seen a paper by Winge (1932), where it is shown that the strigose hairs of *strigosum* are dominant to the patent hairs of ordinary *P. Rhoëas*, and these characters differ by a single factor. The observed ratios generally show an excess of recessives over the expected and a similar condition obtains in the present experiments.

dominance or epistasy over the characters of *P. Rhoeas*. In this respect it is noteworthy that latex colour in *P. Rhoeas* is controlled by more than one factor, and no doubt the same applies to habit and foliage characters. Most likely a similar condition exists in *P. commutatum*, but since these factors are in the homozygous condition they are dominant to those of *P. Rhoeas*.

In conclusion it may be said that the two species are fairly closely related to one another, and since the geographic distribution of one lies within that of the other, they are of recent common origin. In the process of evolution they have been differentiated from one another genetically and cytologically, but not completely, and natural selection has been more rigorous on one than the other.

SUMMARY.

Two forms of *P. commutatum* ($2n = 14$) differing in a group of floral characters are shown to differ by a single factor or by a group of very tightly linked factors.

Strigose hairs are incompletely dominant to patent hairs and are controlled by a single pair of factors which is independent of the factor or factors for the above group of floral characters.

Albinism is recessive and differs from the normal green by a single factor.

Hybrids between *P. Rhoeas* ($2n = 14$, wild and cultivated forms) and *P. commutatum* are described; the characters of *P. commutatum* are mainly dominant or epistatic to those of *P. Rhoeas*.

Consideration of the genetics of the two species, their variation and geographic distribution, and the cytology of the hybrids, leads to the conclusion that they have been recently differentiated from a common origin.

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THE GENETICS OF *PAPAVER RHOEAS* AND RELATED FORMS.

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(With Plate VIII.)

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INTRODUCTION.

THIS study was begun by Mr W. C. F. Newton in 1923, and since his death in 1927 has been continued by the present author. A paper was published (Newton, 1929) describing the material and results up to 1928. In that paper an account was given of the various forms of *P. Rhoeas* L. used in these experiments, namely *P. Rhoeas* and var. *violaceum* Newton, the Shirley poppy, Carter's "Blue Shades" and "Orange Shades," and horticultural forms of *P. Hookeri* Baker (all $2n = 14$). Since then new factors have been studied and further knowledge obtained on certain factors, their interactions and linkage relationships.

Although self-sterility is common, selfed flowers frequently set seed. In one season 132 flowers on 68 plants of wild *P. Rhoeas* and experimental material were selfed. 56 set no seed, 72 set a total of 832 seeds and 4 set 976 seeds (*i.e.* over 100 seeds per capsule). Moreover, protection of a plant by a muslin-covered cage did not always prevent natural crossing from taking place. Consequently from 1929 onwards the flowers used in breeding work have always been protected by pergamine bags, and flowers used as females have always been emasculated.

The descriptive terminology and factor symbols used here are the same as in the previous paper. The phenotypic effects formerly attributed to the factor *r*, however, have been found to be partly incorrect, and hence this factor and the results connected with it should be neglected.

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Source of the flower-colour factors in these experiments.

c was found in the Shirley poppy, in three plants of Carter's "Blue Shades," in Sutton's "Double Strain," and was probably present in one plant from commercial seed. Other forms all carried the dominant allelomorph.

p was introduced from *P. Rhoeas* var. *violaceum* and a plant of *P. Hookeri*.

b was obtained in the Shirley poppy and *P. Hookeri*. The dominant allelomorph occurred in *P. Rhoeas* and var. *violaceum*, *P. Hookeri* and in Carter's "Blue Shades." (*P. Rhoeas* var. *violaceum* was wrongly described in the previous paper, Newton 1929, as having the factor **b**.)

t was found in all forms except *P. Rhoeas* and var. *violaceum*.

W was introduced from a *P. Hookeri* plant and from a plant out of a commercial strain.

F was brought in from two *P. Hookeri* plants.

i occurred in certain Shirley poppies and in *P. Hookeri*. The allelomorphic factor was introduced from *P. Rhoeas* and var. *violaceum*, the Shirley poppy and Carter's "Blue Shades."

e was found in certain Shirley poppies, Carter's "Blue Shades," Sutton's "Double Strain" and in a plant from commercial seed.

THE EFFECT, INTERACTION AND SEGREGATION OF THE FLOWER-COLOUR FACTORS.

The constitution of wild *P. Rhoeas* has been elucidated with regard to the above group of flower-colour factors. *P. Rhoeas* has scarlet petals with a black petal blotch, black filaments, anthers and stigmatic rays, and may be represented factorially by

CC PP BB TT ww ff II EE.

Dominance of these six factors and of **W** and **F** is complete or almost complete over their respective allelomorphs. For convenience *P. Rhoeas* is taken as the type, and in describing any phenotype the only characters mentioned are those in which it differs from *P. Rhoeas*. Similarly, in presenting any genotype, only those factors which differ from the type will be given and only one of each of the pairs of factors, *i.e.* a plant homozygous recessive for a factor will only be given one symbol and heterozygotes will be described like homozygous dominants except in special circumstances where heterozygosity requires to be made clear.

c dilutes and localises the petal colour.

p produces claret petal colour.

b dilutes the petal colour and produces a white petal blotch and tinged filaments, with which are associated yellow anthers and white stigmatic rays.

t converts the petal colour to crimson magenta, probably owing to the absence of a petal pigment.

W produces a white petal edge.

F intensifies the petal colour in **bt** or **et** plants and also localises the petal colour in **bti** or **eti** plants, *i.e.* **F** is a specific modifier of **t**, etc.

i produces flavone, and when anthocyanin is present causes the petal colour to acquire a more bluish shade. i also causes the petals to have a bright sheen.

e in a t plant dilutes and alters the petal colour and produces a brown petal blotch, brown filaments, yellow anthers and brown stigmatic rays.

The various combinations of these factors and their allelomorphs, together with their interactions, give a wide range of forms. The factor W does not have any effect other than that on the edge of the petal and therefore it has been neglected in compiling the following list.

List of the phenotypes and presumed phenotypes for 91 different genotypes out of the possible 128 different combinations of seven pairs of factors.

* indicates the genotypes whose phenotypes have not been ascertained by experiment, but have been arrived at by comparison with other observations of the factor interactions.

Where a phenotype has been illustrated in the previous paper (Newton, 1929) it is referred to by the letter N. followed by the plate and figure number.

+F*	scarlet <i>P. Rhoeas</i> . N. Plate XXIII, fig. 4.
c*, ct, cF*, ctF*	white striated scarlet. N. Plate XXI, figs. 4-6.
p, pF*	claret. N. Plate XXII, fig. 4.
b, bF	paler scarlet than <i>P. Rhoeas</i> , white blotch, tinged filaments, yellow anthers. N. Plate XXIII, fig. 3.
t, tF	crimson magenta. N. Plate XXIV, fig. 1.
et, cet	salmon, brown blotch and filaments, yellow anthers (cet may be pale or white).
i*, iF*	darker form of carmine.
cp*, cpt, cpF*, cpFt*	white striated mauve (also one plant ccPPBbEettIfffww).
cb, cbF*	pale scarlet (paler than b), white blotch, tinged or white filaments, yellow anthers.
pb, pbF	claret, white blotch, tinged filaments, yellow anthers. N. Plate XXII, figs. 2 and 3.
cpb, cpbF*	pale claret (paler than pb), white blotch, tinged or white filaments, yellow anthers.
pi*, piF*	darker form of petunia.
pt, ptF*	rather pale port.
ci*, ciF*	scarlet, netted and flushed white.
cpi*, cpiF*	claret, netted and flushed white.
ti, tiF	crimson or cherry. N. Plate XXIII, fig. 5.
bi	carmine, white blotch, tinged or white filaments, yellow anthers
cbi*	pale carmine, white blotch, tinged or white filaments, yellow anthers.
pbi	petunia, white blotch, tinged or white filaments, yellow anthers.
cpbi*	pale petunia, white blotch, tinged or white filaments, yellow anthers.
bt	rose pink, white blotch and filaments, yellow anthers. N. Plate XXIII, fig. 2.
cbt, cbtF*	pale rose pink (almost indistinguishable from pale pink) or white, white blotch and filaments, yellow anthers. N. Plate XXI, figs. 1 and 3.
pbt	pale mauve, white blotch and filaments, yellow anthers.

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cpbt, cpbtF*	pale mauve or white, white blotch and filaments, yellow anthers.
cpbti	pale mauve or cream, white blotch and filaments, yellow anthers. N. Plate XXI, fig. 2.
bti	pink, white blotch and filaments, yellow anthers. N. Plate XXIII, fig. 1.
cbti	pale pink or cream, white blotch and filaments, yellow anthers.
pbti	mauve, white blotch and filaments, yellow anthers. N. Plate XXII, fig. 1.
cti, ctiF*	crimson or cherry, netted and flushed white. Plate VIII, fig. 7.
pcti, pctiF*	port, netted and flushed white. N. Plate XXV, fig. 1.
pti, ptiF	port. N. Plate XXII, fig. 6.
btF	rosy scarlet, white blotch and filaments, yellow anthers. Plate VIII, fig. 3.
btFi	rose crimson flushed white centre, white blotch and filaments, yellow anthers. N. Plate XXIV, figs. 2 and 3.
bet, cbet	salmon, white blotch and filaments, yellow anthers (cbet may be pale or white). Plate VIII, fig. 2.
pet, cpet	lilac (almost pale mauve), brown blotch and filaments, yellow anthers (cpet may be pale or white).
peti, cpeti	lilac, brown blotch and filaments, yellow anthers (cpeti may be pale). Plate VIII, fig. 4.
eti, ceti	salmon pink, brown blotch and filaments, yellow anthers (ceti may be pale). Plate VIII, fig. 1.
beti, cbeti	salmon pink, white blotch and filaments, yellow anthers (cbeti may be pale).
pbet, cpbet	pale lilac (almost pale mauve), white blotch and filaments, yellow anthers (cpbet may be pale or white).
etF*, cetF	yellowish scarlet (paler than fiery red), brown blotch and filaments, yellow anthers (cetF may be pale).
betF, cbetF	yellowish scarlet (paler than fiery red), white blotch and filaments, yellow anthers (cbetF may be pale).
etiF, cetiF	fiery red, flushed white centre, brown blotch and filaments, yellow anthers (cetiF may be more flushed).
betiF, cbetiF	fiery red, flushed white centre, white blotch and filaments, yellow anthers (cbetiF may be pale). Plate VIII, fig. 5.
petF*, cpetF	almost claret (paler than dull carmine lake), brown blotch and filaments, yellow anthers (cpetF may be pale).
pbetF, cpbetF	almost claret (paler than dull carmine lake), white blotch and filaments, yellow anthers (cpbetF may be pale).
petiF*, cpetiF	dull carmine lake, flushed white centre, brown blotch and filaments (cpetiF may be more flushed).
pbetiF, cpbetiF	dull carmine lake, flushed white centre, white blotch and filaments, yellow anthers (cpbetiF may be more flushed). Plate VIII, fig. 8.
pbtif	dark mauve, flushed white centre, white blotch and filaments, yellow anthers. N. Plate XXIV, figs. 4 and 5.
cbtiF	cream edged rose crimson, white blotch and filaments, yellow anthers. Plate VIII, fig. 6.
cpbtiF	cream edged dark mauve, white blotch and filaments, yellow anthers.
pbeti, cpbeti	lilac, white blotch and filaments, yellow anthers (cpbeti may be pale and indistinguishable from pale mauve or cream).

Table I gives the observed single-factor ratios together with expectation. No significant difference was observed in the results of back-crosses

TABLE I.

Single-factor ratios.

Cross	No. of families	Observed		Calculated		Deviation	Deviation σ
		Dominant	Recessive	Dominant	Recessive		
+c × +c	17	1252	498	1312.5	437.5	+60.5	3.37
	*7	975	393	1026.0	342.0		
+c × cc	3	19	29	24.0	24.0	+5.0	1.44
cc × cc	66	0	2464	0	2464		
	*12	0	573	0	573		
+p × +p	48	3017	1018	3026.25	1008.75	+9.25	0.34
	*7	772	246	763.5	254.5		
+p × pp and reciprocal	58	1959	1800	1879.5	1879.5	-79.5	2.59
	*16	738	631	684.5	684.5		
pp × pp	34	0	1743	0	1743		
	*14	0	1037	0	1037		
+b × +b	40	2121	674	2096.25	698.75	-24.75	1.08
	*2	332	123	341.0	114.0		
+b × bb and reciprocal	27	2151	2110	2130.5	2130.5	-20.5	0.63
	*13	758	686	722.0	722.0		
bb × bb	123	0	4879	0	4879		
	*38	0	894	0	894		
+t × +t	11	758	273	773.25	257.75	+15.25	1.10
	*3	413	160	429.75	143.25		
+t × tt and reciprocal	30	1258	1299	1278.5	1278.5	+20.5	0.81
	*7	534	569	551.5	551.5		
tt × tt	175	3	9386	0	9389		
+W × +W	24	742	333	806.25	268.75	+64.25	4.52
	*1	75	23	73.5	24.5		
+W × ++ and reciprocal	50	1678	1926	1802.0	1802.0	+124.0	4.13
	*17	548	559	553.5	553.5		
++ × ++	75	0	4414	0	4414		
	*10	0	585	0	585		
+F × +F	3	57	23	60.0	20.0	+3.0	0.77
	*2	36	17	39.75	13.25		
+F × ++ and reciprocal	24	1036	1015	1025.5	1025.5	-10.5	0.46
	*5	229	188	208.5	208.5		
++ × ++	107	0	3971	0	3971		
+i × +i	16	693	257	712.5	237.5	+19.5	1.46
+i × ii and reciprocal	16	255	269	262.0	262.0		
						+7.0	0.61
ii × ii	18	0	771	0	771		
+e × +e	20	827	310	852.75	284.25	+25.75	1.93
ee × +e	1	7	9	8.0	8.0		
ee × ee	8	0	166	0	166	+1.0	0.50

* Previously published results which are incorporated in the total results.

which were made reciprocally. Before dealing with each factor individually it may be mentioned that the eight factors fall provisionally into three linkage groups, as follows: (1) **bW**, (2) **pFit**, (3) **ec**.

The factor c.

c in almost all combinations dilutes the petal colour, and in certain cases its effect may be detected in the heterozygous condition. Shirley poppies, a horticultural classification, all carry **c b t**. The petal blotch, filaments and stigmatic rays of **bt** plants are white and the flower colour is diluted. The additional diluting effect of **c** causes the petal colour to be very pale, white or cream, and as a result often makes it impossible to score the factor **p**.

White or white striated forms with a black blotch, from Carter's "Blue Shades," were reported to have given fully or almost fully coloured forms. The latter were cherry or crimson netted and flushed with white at the base of the petals, and had a black blotch. A corresponding **p** form also occurred and is figured in N. Plate XXII, fig. 5. This figure is too pale, owing to bleaching during the hot dry period in July, 1928, and should be of the same intensity as Plate VIII, fig. 7. The genotype of these forms has now been discovered. The white or white striated forms were **ct**, the apparently fully coloured cherry or crimson **cti** and the port **pcti**. **c**, therefore, in **ti** plants, does not cause its typical diluting effect. It is suspected from other evidence that the phenotypes of **c** and **ci** forms are very similar if not identical with those of **ct** and **cti** forms respectively.

The production of striations of colour on the whites with black blotches is variable. On pure whites **p** cannot of course be scored. The striations of colour range from scarlet to a pale mauve or slaty blue, hence the name "Blue Shades." It has been found, however, that these slaty blue types are not necessarily **p**, indeed one distinctly blue plant was discovered to be homozygous for the dominant allelomorph. **c** and **e** are very closely linked, and when **e** was introduced into these experiments it was almost always associated with **c**. Certain families have been grown which theoretically should have contained some cross-over plants containing **e** and the allelomorph of **c**, but these could not be distinguished from **ec** plants. It appears that **c** is variable in its diluting effect in **e** plants and may in some cases have no diluting effect.

In compiling the observed results for the segregation of the factor **c**, families have been omitted where the scoring proved difficult, *e.g.* in families homozygous for **e**. In one family **b** and **cb** plants were omitted, as they were rather difficult to distinguish. The results in Table I show that there are considerable quantitative deviations in the segregation of **c**; an excess of recessives in F_2 's. If, however, two F_2 families which showed a significant deviation from expectation were omitted from

Table I the results would be in accordance with expectation. These families, 23/27 and 9/31, gave respectively the following ratios of dominants and recessives: 173 : 84, 79 : 39.

The factor p.

p causes the petal pigment to be some form of purple instead of red. The effect of **p** can readily be scored in all combinations, with the exception of certain ones involving **c**. These forms have been mentioned already, namely **cbt**, **ct**, probably **c**, and, though rarely, **cet** and **cbet**. Where such forms occurred in families segregating for **p**, all **c** plants were neglected in presenting the results in Table I. The observed F_2 results agree well with expectation, but the back-cross results show some deviation. This is mainly due to the inclusion of the following three families:

	Dominant	Recessive
19/27	146	100
45/28	67	16
9/31	49	29

The factor b.

b dilutes the petal colour and produces a white petal blotch, tinged filaments, yellow anthers and white stigmatic rays. As in the case of **c**, **b** seems to have little or no diluting effect in combination with **e**. Although the filaments are generally tinged with anthocyanin and the blotch is usually white, the filaments may be white and the blotch grey or mauve. Such variation may occur between different flowers on the same plant. The production of the petal blotch, whether black or white, is also variable and it may be entirely absent. This variability is usually more marked as the plants age and seems to be confined to particular lines; the blotch is generally well defined in **i** plants. **b** generally lightens the colour of the seeds.

The multiple effects of the factor **b** and its allelomorph, although variable in their expression in some cases and modified in others by the interaction of the factor **e**, can always be recognised with certainty.

The results in Table I show that **b** behaves as an ordinary Mendelian factor. Three families, 28/29, 29/29 and 29/32, showed a striking deviation from the expected ratio of 3 : 1 and are omitted from Table I. Families 28/29 and 29/29 were raised from reciprocal crosses between a chimerical plant mentioned and figured in the previous publication (p. 401) and a non-chimerical but otherwise identical sister plant. These plants were of the constitution **ccppBbttii**, and the chimerical sectors clearly resulted from a somatic mutation of the dominant allelomorph of **b** to the re-

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cessive condition, since some filaments and regions of the stigmatic rays were non-pigmented. Family 28/29, where the chimerical plant was the female parent, consisted of 64 dominants and no recessives, and family 29/29 consisted of 30 dominants and two recessives. These abnormal results do not appear to be directly connected with the chimerical nature of one of the parent plants. Family 29/32 was an F_2 of *P. Rhoeas* \times **pbt**i and consisted of 44 dominants and no recessives. Family 30/32, however, from the reciprocal cross, consisted of 38 dominants and 10 recessives. In all three abnormal families there has been an elimination of **b** gametes or zygotes, and in family 29/32 this has been confined to one side of the cross. Unfortunately the pollen of the parent plants was not examined and the estimation of zygotic failures is impracticable, hence it cannot be determined whether the lethal is gametic or zygotic.

The factor t.

t has the effect of darkening the petal colour (crimson magenta), and this is probably due to the absence of a pigment (scarlet) produced by its allelomorph. It has been mentioned that **b** plants generally have a tinge of anthocyanin on the filaments, and the petal blotch if present may be white or grey. In **bt** plants the filaments and petal blotch are always white. The effect of **t** is more easily recognised on **b** plants than on plants carrying the allelomorph of **b**. As stated earlier, it is not known definitely whether **c**, **pc**, **ci** and **pci** are phenotypically similar or identical with **ct**, **pct**, **cit** and **pcit** forms respectively. In compiling the results of the segregation of **t**, families in which any of the first group of forms are likely to occur have therefore been omitted.

Six F_2 families have also been omitted from Table I. These F_2 's were from crosses of sister plants of *P. Rhoeas* with the same **t** plant. Three of these families, 26/32, 27/32 and 28/32, of which the first two were reciprocal F_2 's, showed a significant deviation from expectation. Families 29/32 and 30/32 were also reciprocal F_2 's and also showed some deviation from expectation in the same direction as the first three families, namely a deficiency of dominants. In view of these aberrant ratios, all six families were neglected and the results are given below.

Family No.	Dominant	Recessive
26/32	62	36
27/32	62	37
28/32	50	48
29/32	29	15
30/32	31	17
35/31	24	10

The results in Table I show that the genetical behaviour of **t** is in accordance with expectation, with the exception of three unexpected plants containing the dominant allelomorph of **t**. These exceptions will be mentioned later.

The factor W.

W produces a white petal edge. This effect of course cannot be observed on white, cream or pale-coloured flowers, *i.e.* in most **c** plants. In presenting the results for **W**, **c** plants have therefore been omitted. Certain forms, particularly those carrying the allelomorph of **i** and especially in combination with **e**, are prone to bleach at the petal edge, giving an effect like that of **W**. These effects can often be distinguished. **i** plants rarely bleach in this manner. **W** is very variable in its expression. Thus the white edge may be broad and clearly defined, or narrow and merely an ill-defined pale edge. The cause of the variation is not known, but it may be due to modifying factors. Naturally such variation causes difficulty in scoring and in some cases a plant scored as having a plain edge has been found to be genetically **W**. Generally in such cases the progeny consist of plain-edged and narrow or pale-edged plants with a deficiency of the theoretically expected number of plants showing the effect of **W**. In other families, however, the white edge can be scored relatively easily.

The total results of the segregation of **W** in Table I show a significant deviation from expectation, but it is believed that this is largely due to the difficulty of scoring certain families and the irregular expression of the factor **W**.

The factor F.

F has no apparent effect on the wild type. In **bt** plants, however, **F** intensifies the colour and with **i** in addition it also confines the colour to the outer regions of the petals, leaving the centre flushed white. **F** also has its intensifying effect on **te** and **bte** plants.

Since **F** cannot be recognised in plants carrying the allelomorph of either **b** or **t** in combination with the allelomorph of **e**, such plants have been omitted in presenting the results for **F** in Table I. Families from parent plants having **F** and **t** in the linked condition have also been omitted.

The observed results in Table I show clearly that **F** behaves in a normal Mendelian manner.

The factor i.

i causes flavone to be produced, and when anthocyanin is present causes the colour to be more bluish. This factor also produces a sheen on the petals and causes the colour to appear brighter. In Shirley forms where little or no anthocyanin is present, the petals are white except in *i* plants where they are cream. The allelomorph of *i* inhibits the production of flavone, but dominance is not always complete as flavone is usually present near the blotch and may spread to some extent over the petal. It has been mentioned that coloured flowers having the allelomorph of *i* are inclined to sun-bleach at the petal edge. It is also noticeable that the petals of *ct* and *cbt* types in hot sunny conditions often shrivel up to the region where the flavone is present near the blotch, whereas the *cti* and *cbti* types remain quite fresh. It appears that *ti* plants may be crimson or cherry. Cherry is much more bluish than crimson and has a brighter sheen. The difference between the two forms, if genetical, has not been discovered. N. Plate XXIV, fig. 1, was described as a cherry, but it does not give quite the correct impression of the bright waxy sheen, and actually it is a close approximation to the appearance of crimson magenta *t*.

The interactions of *i* and its allelomorph with certain factors has been mentioned. Thus *ct* plants are white striated scarlet or white, and *cti* plants are almost fully coloured. Note the variable production of full colour near the blotch due to the presence of flavone in that region in *ct* plants (N. Plate XXI, figs. 4-6). The white netting on *cti* plants (N. Plate XXV, fig. 1, and Plate VIII, figs. 1 and 7) is probably due to *i* causing the petals to be more stiff, and the angles of the folds in the bud to be more acute, so that the pigment may not be produced along the lines of the folds. *F* only shows its effect in certain combinations. This consists of an intensification of the petal colour and in *i* plants the colour is also localised.

With the above varied expression of *i* and its allelomorph *i* may be scored quite readily. In doubtful cases chemical tests may be employed. In pale-coloured forms such as *b* or *e* plants the presence or absence of flavone may be determined by immersing the petal in an atmosphere of ammonia, when the petal will show a distinct yellow colour where the flavone is located. When the petals are deeply coloured with anthocyanin as in most combinations lacking *b*, and in combinations where *F* has its intensifying and localising effect, it seems that even although the factor *i* is present, very little if any flavone occurs in a form which will

give the yellow colour with ammonia vapour. Here another chemical test may be used with varying success. The outer margin of the petal is crushed with 2 c.c. of $\frac{1}{2}$ per cent. hydrochloric acid and shaken with an equal volume of ethyl acetate. The acetate fraction is then decanted off and shaken with 1 c.c. of sodium carbonate solution. If flavone is present a yellow colour will be obtained. Fortunately most of the deeply coloured forms can be scored for **i** by their visible characters.

In presenting the results in Table I five F_2 families, 26-30/32, have been omitted, since **i** and **t** were completely linked and therefore the same deviation was shown for the segregation of **i** as for **t**. Apart from these families the results are in good agreement with expectation.

The factor e.

The effect of **e** on *P. Rhoeas* is not known, but on **t** plants it produces a brown blotch, brown filaments and stigmatic rays, and yellow anthers; it also dilutes the petal colour. This diluting effect is such that **c** or **b** do not show their normal diluting effect. Thus, for example, **ebt** plants are practically the same colour as **et** plants. For this reason **F** is able to show its effect in a **cetF** plant, which is identical with that of **F** in a **cbetF** plant. **e** also alters the petal colour. In the red series the colour is of a salmon shade and in the **p** series the colour is of a lilac shade with a brownish tint (especially when the flower ages) which is similar to the claret **pb** shade.

As already mentioned, a **ct** plant commonly has the petals striated with colour. The effect of **e** on such a plant is to remove the striations, leaving the petal white or uniformly but palely coloured. Another effect of **e** is that it causes the seeds to be rather pale yellowish.

In some families **cb** plants were difficult to distinguish from **cbe** plants, owing to the colour being extremely pale. In presenting the results for **e** these families have been omitted, or else only plants in which **b** was absent have been considered.

Table I shows that the genetical behaviour of **e** is in agreement with expectation.

LINKAGE.

In compiling the observed results given in Tables II and III, families or certain classes in some families were omitted where the scoring of a particular character was very difficult or impossible.

These tables show clearly that the eight factors fall provisionally into three linkage groups: (1) **bW**, (2) **pFit**, (3) **ec**. In **c** plants it is generally difficult to recognise the effects of the other factors and thus it is difficult to show directly the linkage relationship of **c** to all other factors except **e**.

TABLE II.

Independent factor segregation.

Cross	No. of families	Observed				Calculated				X ²	P
		XY	Xy	xY	xy	XY	Xy	xY	xy		
$\frac{++}{pe} \times \frac{++}{pe}$	1	56	11	19	7	51.3	17.4	17.4	5.8	3.1810	0.3
$\frac{+e}{p+} \times \frac{+e}{p+}$	12	388	181	152	32	423.5	141.2	141.2	47.1	19.864	<0.01
$\frac{+p+e}{p+} \times \frac{+e}{p+}$	1	62	20	20	2	52.5	17.5	17.5	6.5	5.5494	0.10
$\frac{F+}{+e} \times \frac{++}{+e}$	2	65	18	72	31	69.75	23.25	69.75	23.25	4.1651	0.20
$\frac{+e}{i+} \times \frac{+e}{i+}$	6	236	103	104	29	265.5	88.5	88.5	29.5	9.2152	0.02
$\frac{ii+e}{+e} \times \frac{+i+e}{+e}$ and reciprocal	4	35	13	25	8	30.4	10.1	30.4	10.1	2.9249	0.50
$\frac{++}{be} \times \frac{++}{be}$	2	51	18	17	8	52.8	17.6	17.6	5.9	0.8380	0.80
$\frac{+e}{b+} \times \frac{+e}{b+}$	1	58	16	17	2	52.3	17.4	17.4	5.8	3.2331	0.30
$\frac{++}{be} \times \frac{b+}{be}$	1	15	8	26	8	21.4	7.1	21.4	7.1	3.1313	0.30
$\frac{We}{+e} \times \frac{We}{+e}$	4	92	32	36	14	97.8	32.6	32.6	10.9	1.5934	0.70
$\frac{++}{We} \times \frac{++}{+e}$	1	25	19	34	7	31.9	10.6	31.9	10.6	9.5112	0.02
$\frac{++}{pb} \times \frac{++}{pb}$	10	369	87	151	45	366.75	122.25	122.25	40.75	17.3831	<0.01
$\frac{+b}{p+} \times \frac{+b}{p+}$	3	107	35	36	9	105.2	35.1	35.1	11.7	0.6770	0.90
$\frac{p+}{+b} \times \frac{+b}{pb}$	2	45	117	13	34	78.4	78.4	26.1	26.1	42.199	<0.01
$\frac{+pbb}{p+} \times \frac{+p+b}{p+}$	2	97	93	30	36	96.0	96.0	32.0	32.0	0.7291	0.90
$\frac{p+}{pb} \times \frac{++}{pb}$	1	32	5	21	1	22.1	7.4	22.1	7.4	10.824	0.01
$\frac{++}{pb} \times \frac{pb}{pb}$ and reciprocal	3	81	65	83	48	69.25	69.25	69.25	69.25	11.5084	0.01
$\frac{+b}{p+} \times \frac{pb}{pb}$	1	18	14	21	10	15.75	15.75	15.75	15.75	4.3649	0.20
$\frac{+p+b}{p+} \times \frac{ppbb}{p+}$ and reciprocal	2	14	14	20	14	15.5	15.5	15.5	15.5	1.7416	0.70
$\frac{++}{ib} \times \frac{++}{ib}$	4	164	41	108	30	193.0	64.3	64.3	21.4	33.918	<0.01
$\frac{+b}{i+} \times \frac{+b}{i+}$	2	56	17	13	8	52.8	17.6	17.6	5.9	2.1636	0.50
$\frac{+i+b}{+b} \times \frac{+i+b}{+b}$	1	17	2	7	2	15.75	5.25	5.25	1.75	2.730	0.50
$\frac{+i+b}{tb} \times \frac{+ibb}{tb}$	1	3	5	0	1	3.4	3.4	1.1	1.1	1.9088	0.50
$\frac{++}{tb} \times \frac{++}{tb}$	8	438	130	195	69	468.0	156.0	156.0	52.0	21.565	<0.01
$\frac{+t+b}{+b} \times \frac{+tbb}{+b}$	2	41	50	37	41	42.25	42.25	42.25	42.25	2.1483	0.50

e is closely linked with **c**, and **e** is shown to be independent of the factors **F**, **i**, **b** and **W**. It follows therefore that **c** is also independent of these factors. The relationship of **e** and **t** has not been tested, but **t** is closely linked with **F** and **i** and hence is independent of **e** and **c**.

In Table II it will be observed that the combined results for **e** and **p** suggest the possibility of linkage. In the coupled condition the cross-over percentage is 41.5 per cent. and in repulsion it is 39 per cent. These results are from F_2 's and therefore cannot be considered as conclusive evidence of linkage, but back-crosses are being made to settle this point. Until these results are forthcoming it is regarded as advisable to consider that the factors fall into three linkage groups. If **e** and **p** are linked it is probable that **e** lies on the opposite side of **p** from **Fi** since **F** and **i** are closely linked and are about 16 units from **p**, and show independence of **e**.

The results in Table II also show that **b** is independent of **p**, **i** and **t**. **b** and **W** are linked, therefore **W** is also independent of these factors. It is only possible to study directly the relationship of **b** and **F** in **e** plants and such a test has not been effected. **F** is linked with **p**, **i** and **t**, however, and thus is independent of **b** and **W**.

The F_2 results for **ib** and **tb** show a striking deviation from expectation. It will be remembered that six F_2 families from wild *Rhoeas* were omitted from Table I because of their aberrant segregation of **t** and **i**. Five of the eight families segregating for **tb** and all four segregating for **ib** were members of these six families. This deviation therefore is mainly due to the aberrant single factor segregation of **i** and **t**. Two groups of results for **pb** also show considerable deviation, but the remainder agree on the whole with expectation.

Cross-over percentages have been calculated by the product ratio method (Fisher and Balmukand, 1928) except in the case of back-crosses.

The results in Table III show that **b** and **W** are closely linked. Here the cross-over percentage may be taken to be about 1 per cent., as the back-cross results with one exception, 10/30, are close to this figure. The F_2 results show an increase in the cross-over percentage, namely 10.5.

The back-cross results for **p** and **F** show the cross-over percentage to be 16 per cent. The results from other crosses show a decrease in the cross-over percentage, and those of 1/32 and 2/32 will be discussed later.

No back-cross data have been obtained on the linkage of **p** and **i**, but the F_2 results are fairly constant and show a cross-over percentage of 16 per cent. Families 1/32 and 2/32 again show a significant deviation as they do in the case of the **pF** results.

The percentage crossing-over between **p** and **t** in the different crosses

TABLE III.

Linkage results.

Cross	Family No.	+W	++	bW	b+	Cross-over percentage
$\frac{+W}{b+} \times \frac{b+}{b+}$	10/30	26	0	5	10	
	15/31	38	1	0	24	
	Total	64	1	5	34	5.8
	Expectation	49.0	3.0	3.0	49.0	
$\frac{b+}{b+} \times \frac{++}{bW}$	12/29	1	83	45	0	
	5/30	0	76	72	2	
	6/30	1	113	168	0	
	7/30	0	69	61	1	
	11/30	1	50	54	1	
	Total	3	391	400	4	0.9
	Expectation	3.5	395.5	395.5	3.5	
$\frac{++}{bW} \times \frac{++}{bW}$	33/29	10	12	13	0	
	35/29	28	24	6	1	
	58/29	77	46	8	0	
	62/29	11	16	19	0	
	63/29	30	5	14	0	
	71/29	11	7	8	0	
	Total	167	110	68	1	10.5
	Expectation	173.9	85.5	85.5	0.9	
$\frac{+F}{p+} \times \frac{p+}{p+}$	6/30	65	11	19	68	
	15/31	10	4	1	9	
	53/32	18	4	0	22	
$\frac{p+}{p+} \times \frac{+F}{p+}$	8/28	38	4	8	24	
	25/28	44	0	3	40	
	10/29	80	16	7	79	
	3/30	54	6	29	35	
	Total	309	45	67	277	16.0
	Expectation	293.0	56.0	56.0	293.0	
$\frac{++}{pF} \times \frac{p+}{p+}$	9/31	8	41	27	2	12.8
	Expectation	5.0	34.0	34.0	5.0	
$\frac{+F}{p+} \times \frac{++}{p+}$	4/30	140	96	10	66	
	5/30	27	23	2	21	
$\frac{++}{p+} \times \frac{+F}{p+}$	22/28	34	15	1	18	
	52/28	26	6	5	47	
	Total	227	140	18	152	11.0
	Expectation	253.9	149.0	14.7	119.4	
$\frac{++}{pF} \times \frac{++}{p+}$	2/32	16	49	24	0	
$\frac{++}{p+} \times \frac{++}{pF}$	1/32	24	52	19	2	
	Total	40	101	43	2	3.3
	Expectation	48.1	91.4	44.9	1.5	

TABLE III (continued).

Linkage results.

Cross	Family No.	++	+i	p+	pi	Cross-over percentage
$\frac{++}{p\ i} \times \frac{++}{p\ i}$	4/32	65	9	9	19	
	6/32	35	3	4	5	
	7/32	32	5	2	8	
	26/32	55	7	7	29	
	27/32	55	12	7	25	
	28/32	45	14	5	34	
	29/32	27	1	2	14	
	30/32	28	5	3	12	
	Total	342	56	39	146	16.0
	Expectation	394.0	42.8	42.8	103.0	
	2/32	59	6	2	22	
	1/32	73	3	2	19	
	Total	132	9	4	41	6.5
	Expectation	133.5	5.9	5.9	40.7	
		++	++	p+	pt	
$\frac{++}{p\ t} \times \frac{pt}{p\ t}$	1/30	9	1	1	12	
	2/31	68	12	16	52	
	24/31	14	3	7	20	
	26/31	8	2	1	11	
	27/31	24	10	5	29	
$\frac{pt}{p\ t} \times \frac{++}{p\ t}$	2/30	68	8	7	79	
	Total	191	36	37	203	15.6
	Expectation	197.0	36.5	36.5	197.0	
$\frac{++}{p\ +} \times \frac{pt}{p\ +}$	9/29	17	108	107	25	
	15/30	9	24	19	8	
	3/31	0	15	12	2	
	6/31	1	7	3	0	
	20/31	5	26	27	4	
$\frac{pt}{p\ t} \times \frac{++}{p\ +}$	8/29	3	11	18	1	
	4/31	0	10	12	2	
	5/31	2	4	5	2	
	Total	37	205	203	44	16.5
	Expectation	40.5	204.0	204.0	40.5	
$\frac{++}{p\ t} \times \frac{++}{p\ t}$	41/29	18	1	2	5	
	35/31	24	5	0	5	
	11/32	46	9	8	12	
	26/32	55	7	7	29	
	27/32	55	12	7	25	
	28/32	45	14	5	34	
	29/32	27	1	2	14	
	30/32	28	5	3	12	
	Total	298	54	34	136	16.0
	Expectation	353.8	38.4	38.4	92.0	
$\frac{++}{p\ +} \times \frac{++}{p\ +}$	12/30	51	29	19	1	20.5
	Expectation	51.0	24.0	24.0	1.0	

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TABLE III (continued).

Linkage results.									
Cross	Family No.	++	+t	p+	pt	Cross-over percentage			
$\frac{++}{p\ t} \times \frac{++}{p\ t}$	8/30	9	8	2	10	20.8			
	Expectation	13	8.7	1.5	5.7				
$\frac{p\ +}{p\ t} \times \frac{+t}{p\ +}$	41/28	17	6	23	2				
	13/30	31	14	40	3				
	Total	48	20	63	55	21.8			
	Expectation	41.1	26.6	60.9	7.4				
		F+	Fi	++	+i				
$\frac{F\ i}{++} \times \frac{++}{++}$	2/32	13	27	48	1				
$\frac{++}{++} \times \frac{F\ i}{++}$	1/32	20	22	55	0				
	Total	33	49	103	1	1.2			
	Expectation	47.0	46.0	92.4	0.6				
		++	+i	t+	ti				
$\frac{++}{t\ i} \times \frac{ti}{ti}$	39/28	45	2	37		4.4			
		++	+c	e+	ce				
$\frac{++}{e\ c} \times \frac{++}{e\ c}$	34/32	45	1	30					
	35/32	36	1	26					
	39/32	59	0	16					
	Total	140	2	72		Approximately 1			
		+++	pFi	+Fi	p++	++i	pF+	F+	p+i
$\frac{+++}{p\ +\ i} \times \frac{+++}{p\ F\ i}$	1/32	53	19	3	2	0	0	20	0
$\frac{+++}{p\ F\ i} \times \frac{+++}{p\ +\ i}$	2/32	48	22	5	0	1	2	11	0
	Total	101	41	8	2	1	2	31	0
	Expectation	91.2	41.6	4.4	1.5	0.5	3.4	43.3	0.02

is relatively constant. Considering only the back-cross and F_2 results, the cross-over percentage is about 16 per cent. The remaining crosses show an increase to 21.8 per cent., but these are probably less accurate than the other results.

Only two families have been obtained which showed linkage between **F** and **i**, and the percentage of crossing-over was 1.2 per cent.

The F_1 of *P. Rhoeas* var. *violaceum* \times **pbti** was back-crossed to the recessive and gave family 39/28. Here the segregation of **i** was not recorded in **t** plants and the cross-over percentage between **t** and **i** was 4.4 per cent. No crossing-over has been observed however between these two factors in 387 F_2 plants from the cross *P. Rhoeas* \times **pbti**.

The cross-over percentage between **e** and **c** is approximately 1 per cent. Unfortunately two of the classes cannot be distinguished with certainty, but back-cross results are being obtained which will provide more accurate figures. It appears from the results in Table III that there is no difference in crossing-over on the male and female sides of the plant.

In the second linkage group the percentage crossing-over between **p** and the other factors is about 16 per cent. in each case. One three-point experiment was made with the factors **pFi**, and the linkage results of these two families, 1/32 and 2/32, have been kept separate in Table III. Here the percentage crossing-over between **F** and **i** is 1.2 per cent., which is in accordance with the above results. The cross-over percentage between **p** and **F** and **p** and **i** however shows a striking decrease, namely from about 16 to 3.3 and 6.5 per cent. respectively. Using these values in calculating expectation in the three-point experiment the close fit of observed with expected figures shows clearly that the order of the factors is **pFi**. The exact locus of **t** is not known, but it is close to **Fi**, since the cross-over percentage between **i** and **t** is 4.4 per cent. or less. The degree of linkage between **F** and **t** has not been ascertained, since the effect of **F** is masked when the allelomorph of **t** is present.

The data in connection with families 1/32 and 2/32 where the decrease in crossing-over occurred are of interest. The families were F_2 's from a plant $6^{242}/30$ of a commercial strain crossed with plant $3^{121}/30$ of experimental material. The second chromosomes of these plants were of the constitution $\frac{+++t}{+++t}$ and $\frac{pFit}{p+it}$ respectively. Family 3/30 showed 28.2 per cent. of crossing-over between **p** and **F**. Plant $6^{242}/30$ was also crossed with plants $3^{124}/30$ and $7^4/30$ whose second chromosomes were of the constitution $\frac{p+it}{p+it}$ and the respective F_2 's showed 18.5 and 17.5 per cent. crossing-over between **p** and **i**. Plant $7^4/30$ was a descendant of a line which gave 12.6 per cent. crossing-over between **p** and **F**.

This evidence suggests that the second chromosomes of the plants crossed on to the plant $6^{242}/30$ were normal, and yet it seems that the introduction of **F** was associated with the reduction in crossing-over. (Note the crosses giving 1/32 and 2/32 were factorially reciprocal, but different F_1 plants were used.)

Another peculiar feature is that the three plants mentioned earlier, which unexpectedly carried the dominant allelomorph of **t**, were descendants of plant $6^{242}/30$. One plant occurred in the F_1 of seven plants

from $6^{242}/30 \times 3^{105}/30 \frac{p+it}{p+it}$. The other two exceptional plants occurred in families 1/32 and 2/32, and each carried **F**. Apart from these three families, 172 families comprising over 900 plants have not contained plants unexpectedly carrying the allelomorph of **t**.

At present the evidence suggests the possibility of chromosome aberration having occurred, *e.g.* by inversion of a piece of the second chromosome, and further investigation is being made on this line.

OTHER FACTORS.

White margin on black petal blotch.

The black petal blotch in wild *P. Rhoeas* and the cultivated forms may have a white margin of varying width (N. Plate XXI, fig. 6; XXII, fig. 6; XXIII, fig. 5; XXIV, fig. 1; XXV, fig. 1; and the present Plate VIII, figs. 1, 4 and 7). An attempt to study the inheritance of this character has been hampered by the fact that it is confined in its expression to plants with a black or brown blotch, that the blotch is frequently not produced or only partly produced, in which case the white margin cannot be studied; or the white margin may be extremely faint, as in Plate VIII, fig. 7, and N. Plate XXI, fig. 6. In the analysis of the results in the table below, those plants with the black blotch undeveloped or partly developed have been neglected.

	No. of families	Observed		Calculated	
		White margin	No margin	White margin	No margin
Back-cross	8	158	184	171	171
F_2	10	316	130	334.5	111.5
One parent homozygous for white margin	21	642	28	670	0

Having in mind the practical difficulties with regard to this character, the results given above cannot be considered as strictly accurate. Moreover, the factorial constitution of some of the plants has not been proved.

It is clear, however, that the presence of a white margin to the black blotch is dominant to its absence. It appears also that the absence of a white margin is primarily due to a single factor **m**. On this interpretation the results are fairly close to expectation, but it is obvious that there is an excess of recessives in the back-cross and F_2 's and there are unexpected recessives in the progeny of crosses where one parent at least was homozygous for the dominant. This seems to be due to the dominant character failing to express itself and is corroborated by the fact that plants with a

black blotch and no white margin have proved to be carrying the dominant factors, and conversely plants with a white margin have occasionally given very few or no plants with white margins in their progeny.

One group of results from wild *P. Rhoeas* has not been included in the table. They show apparently discrepant results on the one hand and orthodox ones on the other. *P. Rhoeas*, 56/29, from one capsule of natural seed, contained 28 plants with the black blotch only slightly developed or undeveloped, seven plants with a white margin to the blotch and 15 plants with no margin to the blotch. Three plants of 56/29 were crossed with a **pbt**i plant which from other evidence presumably had **m** present. One plant, 56⁴³/29, with a white margin, gave an F_1 of 41 plants with the black blotch slightly developed or absent and 31 with a black blotch and no white margin. Reciprocal F_2 's consisted of 24 with the black blotch absent or slightly developed, eight with a white margin and 50 with no white margin.

Plant 56¹⁷/29 had only a faintly developed black blotch and the F_1 consisted of 12 with the black blotch not shown or only partly developed, 35 with a white margin and 40 with no white margin. Reciprocal F_2 's from two white-margined F_1 plants consisted only of plants with the black blotch clearly produced. 112 plants had a white margin and 41 had no white margin.

Plant 56³⁰/29 had the black blotch faintly produced and gave in the F_1 , 47 plants with no black blotch or only slightly developed, no plants with a white margin and 41 with no white margin. The F_2 consisted of two plants with the black blotch faintly developed, no plants with a white margin and 108 plants with no white margin.

As suggested above, the results indicate that the dominant character may not always express itself, and it is possible that there may be other factors which modify the expression of the principal factor. This is more than likely, since the width of the white margin varies and in certain families a particular width may be almost constant.

In the F_2 from plant 56¹⁷/29 the black blotch and white margin were very clearly defined, and the results show that the factor **m** is independent of the factor **p**.

	++	+m	p+	pm
Observed	72	29	40	12
Expectation	86.0	28.7	28.7	9.6

Hair colour.

P. Rhoeas and its cultivated forms have hairs on the flower-bud and on the stem, which may or may not be pigmented. The hairs may be coloured red, brownish red or golden yellow. The degree of pigmentation in the hairs is also variable in different plants. Thus the hairs may only be faintly tinged red at the tip, and grades extend up to the form where the hairs are completely red.

When this character was first studied in experimental material the results showed that red hairs on bud or stem or both were dominant to the other forms (grouped as white), and further that the white hairs were due to a single factor *s* which was tightly linked with the factor *b*. Back-crosses were made and the single-factor ratios and linkage results are given in Table IV. These results show that *b* and *s* are two tightly linked

TABLE IV.

Cross	No. of families	Observed		Calculated					
		Dominant	Recessive	Dominant	Recessive				
$+s \times ss$ and reciprocal	10	526	553	539.5	539.5				
$ss \times ss$	27	0	1552	0	1552				
Cross	Family No.	++	+s	b+	bs	Cross-over percentage			
$\frac{++}{b\ s} \times \frac{bs}{bs}$	10/30	29	2	0	17				
	15/31	39	0	1	23				
$\frac{bs}{bs} \times \frac{++}{b\ s}$	11/29	66	2	1	17				
	12/29	71	3	0	48				
	5/30	74	2	3	71				
	6/30	103	11	5	162				
	7/30	69	0	0	62				
	11/30	47	0	2	47				
Total		498	20	12	447	3.3			
Expectation		472.5	16.0	16.0	472.5				
		W+	Ws	++	+s				
$\frac{+s}{+s} \times \frac{++}{Ws}$	5/30	3	69	74	4				
	6/30	6	162	102	11				
	7/30	0	61	69	1				
Total		9	292	245	16	4.5			
Expectation		12.7	268.4	268.4	12.7				
$\frac{W+}{+s} \times \frac{+s}{++}$	15/31	38	0	2	23	3.2			
Expectation		30.5	1.0	1.0	30.5				
Cross	Family No.	+++	Wbs	+bs	W++	++s	Wb+	+b+	W+s
$\frac{+bs}{+bs} \times \frac{+++}{Wb\ s}$	5/30	74	69	2	0	2	3	0	0
	6/30	102	162	0	1	11	5	0	0
	7/30	69	61	1	0	0	0	0	0
Total		245	292	3	1	13	8	0	0
Expectation		268.6	268.6	1.89	1.89	10.32	10.32	0.07	0.07

factors and that the cross-overs are not merely due to variation in physiological expression of hair colour. Final proof of course would be provided by obtaining a family all of which were of the constitution of a cross-over. The results of the three-point experiment show that *s* lies further from *W* than *b* does.

It was mentioned that variation in hair colour occurred in wild *P. Rhoeas*. When, however, *P. Rhoeas* and some new cultivated strains were crossed with experimental material the following results were obtained:

Family No.	Cross	Red hairs	White hairs
56/29	Natural seed from one capsule of <i>P. Rhoeas</i>	38	12
40/30	56 ¹⁰ /29 red × 20 ¹³ /29 white	79	5
39/30	56 ⁴³ /29 red × 10 ¹ /29 white	41	47
37/30	56 ¹⁷ /29 white × 10 ¹ /29 white	33	50
38/30	56 ³⁰ /29 white × 10 ¹ /29 white	35	51
43/30	101 ¹⁵ /29 red × 10 ¹ /29 white	9	0
44/30	102 ¹ /29 white × 10 ¹ /29 white	24	56
37/31	40 ⁹⁰ /30 white × 40 ⁶² /30 red	71	22

Plants 10¹/29 and 20¹³/29 were known from other crosses to be carrying *s* and therefore these results are not in accordance with those given previously. The evidence suggests that in wild *P. Rhoeas* two factors are concerned with hair colour and that in the experimental material one of these factors was in the homozygous recessive condition.

The distribution of the pigment in the hairs on the bud or on the stem is probably controlled genetically, but the evidence is at present too scanty to say more than this.

Doublelessness.

The material used in these experiments was essentially single-flowered, but plants bearing slightly double flowers occurred sporadically. On one occasion a single-flowered plant of a commercial strain was noted to produce a branch having a fully double flower.

Commercial strains of fully double forms are available, and when two fully double plants were crossed all the progeny 46/30 were fully double. A fully double plant was also crossed with a single-flowered plant from a line which had bred true for singleness for several generations, and the following results were obtained in the *F*₁:

Family No.	Single	Very slight double	Slight double	Double	Full double
45/30	32	2	30	54	0

Out of a total of 118 plants, 32 were single like the single parent and none was fully double like the other parent. The above attempt was made to

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grade the degrees of doubleness, but actually the range was continuous and variation on the same plant was quite considerable.

A single-flowered F_1 plant, from a cross between a fully double plant and a single plant a few of whose sister plants showed a trace of doubleness, was crossed with two single plants from two different single lines and gave the following two families where variation took place as in family 45/30:

Family No.	Single	Slight double	Double	Full double
45/31	17	6	7	0
46/31	22	5	3	0

These and the previous results indicate that singleness is probably incompletely dominant to doubleness and that the inheritance of doubleness is controlled by several factors.

Albinism.

Albino seedlings occurred in one family descended from *P. Hookeri* and *P. Rhoeas* var. *violaceum*. They were also found in six F_3 families from a cross between *P. Rhoeas* and a Shirley poppy. In the latter case the evidence is not conclusive but indicates that albinism was introduced by the wild parent.

The following results are close enough to expectation to conclude that albinism is due to a single factor:

	Green	Albino	Deviation	Deviation/ σ
Seven families	496	120	-34	3.16
Expectation	462	154		

Other evidence also indicates that the factor for albinism is independent of the factors for flower colour.

Latex colour.

The colour of the latex in this experimental material forms almost a continuous series from white to deep yellow or orange. In wild *P. Rhoeas* so far as the author has observed the colour is usually white, but is occasionally very pale yellow.

The cross white \times white always bred true, but it was observed that in one line all plants homozygous recessive for **p** had creamy white latex, whereas all plants carrying the dominant allelomorph had pure white latex. One plant from a family which was homozygous for **p** and bred true for deep yellow latex was crossed reciprocally with a plant heterozygous for **p** from the above line having pure white latex.

The F_1 consisted of 28 plants heterozygous for **p** all having pale yellow latex and 24 plants homozygous for **p**, of which 18 had yellow

latex, 4 had yellow tending towards pale yellow and two tended towards deep yellow.

From these two apparently homozygous lines and the F_1 between them it was therefore possible to make four colour classifications. As will be seen in the F_2 a further classification, very pale yellow, had to be made. A colour chart of these four grades of yellow was made and covered by a piece of celluloid. In this way the latex could be put on the celluloid and the colours compared. As already mentioned the colour variation was almost continuous, and thus a strictly accurate classification was impossible. Moreover, on exposure to air the latex colour darkened rather quickly and the matching of the colour had to be done rapidly. Some whites turned pink and yellows darkened to a reddish brown colour.

By crossing two F_1 plants which were heterozygous for **p** an F_2 was obtained which gave the following result:

Deep yellow		Yellow		Pale yellow		Very pale yellow		White	
P	p	P	p	P	p	P	p	P	p
1	6	1	11	99	11	14	3	24	2

From the above results it is clear that white is recessive to yellow and that the factor **p** has the effect of darkening the latex colour. It is also evident that more than one factor, probably two, controls the inheritance of latex colour, and that **p** might be linked to one of these factors. It may be that the white parent was of the constitution $y_1y_1y_2y_2$ and the deep yellow parent $Y_1Y_1Y_2Y_2$, while the two F_1 plants which were intercrossed were perhaps $Y_1y_1Y_2y_2$ and $y_1y_1Y_2y_2$. The ratio in F_2 of yellow in its different intensities to white would therefore be 7 : 1. The observed is 146 : 26 and the expected is 150.5 : 21.5, which is quite a good fit.

The attempt to explain the different classes of yellow in the F_2 is almost impossible when one bears in mind the effect of **p** and the fact that the phenotypic expression of a particular genotype may not be constant. The latter is suggested by the slight variation in the latex colour of the F_1 **p** plants. It is also possible that the two factors may differ in their degree of expression.

A few F_2 plants were tested in F_3 and the following are the results. Two very pale yellows were crossed with the same white and gave the following two families:

	Deep yellow		Yellow		Pale yellow		Very pale yellow		White	
	P	p	P	p	P	p	P	p	P	p
13/32	0	0	0	4	37	4	15	0	22	7
14/32	0	0	1	0	10	0	2	0	12	0

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The very pale yellow parent of family 14/32 was also crossed with a pale yellow plant and with a deep yellow **p** plant and gave families 16/32 and 15/32 respectively:

	Deep yellow		Yellow		Pale yellow		Very pale yellow		White	
	P	p	P	p	P	p	P	p	P	p
16/32	0	0	16	0	32	0	1	0	0	0
15/32	0	0	10	0	13	0	14	0	0	0

These F_3 results do not throw much light on the problem, but they show some degree of order in that a greater proportion of plants swing in the direction of the intensely coloured latex as the parents involved are further removed from white.

Another group of results showing the inheritance of latex colour, primarily introduced from one **p** plant 10¹/29 with pale yellow latex, is given in Table V. Here the individual ratios are variable, but on the whole they indicate that plant 10¹/29 with pale yellow latex differed from the white by a single factor. On this assumption it may be noted that there is a considerable excess of whites over the expected¹. The results

TABLE V.

Family No.	White	Pale yellow	*Pale yellow	White
37/30 + 38/31	56 ¹⁷ /29 × 10 ¹ /29	44	42	
38/30 + 39/31	56 ²⁰ /29 × 10 ¹ /29	36	53	
39/30 + 40/31	56 ⁴³ /29 × 10 ¹ /29	32	59	
44/30 + 43/31	102 ¹ /29 × 10 ¹ /29	37	43	
15/30	36 ¹ /29 × 10 ¹ /29	17	43	
	Total	166	240	
27/32	38 ²² /31 × 38 ¹⁷ /31	46	53	
28/32	39 ² /31 × 39 ⁷ /31	62	25	
29/32	40 ¹ /31 × 40 ²⁰ /31	19	25	
	Pale yellow			
26/32	38 ¹⁷ /31 × 38 ²² /31	27	69	
35/31	38 ¹⁸ /30 × 38 ⁵ /30	9	27	
30/32	40 ²⁰ /31 × 40 ¹ /31	23	25	
	Total	186	224	
	White			
25/31	15 ⁶ /30 × 3 ¹⁰⁵ /30	0	43	
28/31	15 ⁴⁵ /30 × 6 ²⁴² /30	0	39	
42/31	44 ¹⁷ /30 × 44 ¹⁹ /30	0	10	
	Total	0	92	

* Only families 37/30, 38/30, 39/30 and 44/30 had the yellow latex colour uniformly pale yellow, and in these families no **p** plants occurred. In the other families, where yellow latex occurred, the colour was mainly pale yellow, but there was some variation and **p** plants were found. Family 56/29 was wild *P. Rhoeas*.

¹ These results for latex colour are parallel to those for hair form obtained by Winge (1932), in that the recessive type is the common form in nature and usually occurs in excess of the expected in experiments.

(not shown) also indicate that *p* is not linked with this factor for latex colour.

In conclusion it may be said that white latex is recessive to coloured latex and differs from it probably by two factors. The study of latex colour is complicated by the effect of *p*, and it is clear that more informative results will be obtained by breeding from plants homozygous for *p* or alternatively the dominant allelomorph.

Habit.

The foliage and general habit of *P. Rhoëas* and its various forms show a wide range of variation which is almost continuous and very difficult to classify. Thus the leaf shape varies from very slightly serrated and almost strap-shaped to very much serrated and almost fern-like types. Moreover, the leaf shape usually varies on the same plant, the later developed leaves being different from the earlier ones. It is probable that the strap-shaped leaf type is a recessive form.

Various shades of green also occur, but this has not been studied. The leaves may have a dull glaucous surface or a bright surface. Here again variation takes place on the same plant, and when the plants are attacked by aphid a substance is produced on the leaves which makes them appear glossy and hence renders classification even more difficult. The bright surface is probably recessive.

The habit is generally erect, but forms occur which are dwarf and spreading, with the side shoots coming away from the main shoot almost at a right angle. The lower shoots are ascending—spreading along the ground before bending upwards at the end. The prostrate forms are found occasionally in families which are mainly erect, and it is likely that they are recessive.

There is little doubt that each of the above characters is controlled genetically by more than one factor, and there are probably several modifying factors. It is particularly noticeable that after inbreeding for a few generations the general habit becomes remarkably uniform, but to attempt to describe the differences between two apparently uniform families is usually impossible.

DISCUSSION.

The foregoing results show that in *P. Rhoëas* and its derivatives some deviation from expectation takes place in the segregation of several of the factors. The most striking is that of the factor *W*. As already pointed

out, however, the factor **W** does not always express itself clearly, and the scoring is often complicated by the occurrence of spurious white edges due to sun-bleaching or physiological conditions. Irregular results of the same nature were obtained by Shull (1912), and those of Negodi (1932) and Béguinot (1928) suggest that similar difficulties were encountered. In several families the white edge to the petal was always distinct, and segregation was in accordance with the view that this character differed from the wild by a single factor. The extreme deviation from expectation for this factor, therefore, may be accounted for mainly by its variation in expression which may be caused by modifying factors.

The factor **c** showed some deviation from expectation, but as already mentioned this was largely due to the inclusion of two families which showed extreme deviation. Similarly certain members of a group of F_2 families from wild *P. Rhoeas* showed a significant deficiency of plants having the dominant allelomorphs of **t** and **i**, and thus the whole group were eliminated from the total results for **t** and **i** in Table I. Several of these families were included in Table II in order to demonstrate the independence of **i** and **b** and of **t** and **b**, and no doubt the deviation there exhibited is a result of the abnormal segregation of the factors **t** and **i**.

Three F_2 families showed complete or almost complete elimination of recessive **b** plants and were therefore omitted from Table I. These results show that in some cases at least a lethal or sub-lethal factor is associated with the recessive factor **b**. It is also possible that some of the *Rhoeas* parents of the F_2 's mentioned above had a sub-lethal associated with the dominant allelomorphs of **t** and **i**. This evidence substantiates the view of the author that there are probably several sub-lethal factors present in the experimental material which tend to upset the ratios to a greater or less extent.

Male sterility (contabescence), though variable on the same plant, has also been observed, and inbreeding for a few generations has usually been accompanied by general sterility; failure to set seed even when open to natural cross-pollination was not uncommon.

The frequent occurrence of somatic mutations giving rise to chimerical petals has helped in confirming the effects of the various factors and, for breeding purposes, in determining a plant in an F_2 which was heterozygous for a particular factor. These chimeras have been observed for all the flower-colour factors except **e**. In every case except one the mutation took place from the dominant condition to the recessive condition. The factor **i** was observed to have mutated to the dominant condition on one occasion.

Shull (1912) made crosses between Shirley, white striated, and fully coloured forms. His results agree with those obtained here and can be interpreted on the same basis, showing particularly the interaction of the factors **c**, **b** and **i** and their allelomorphs.

Rasmuson (1920) studied the progeny of crosses between cultivated forms of *Rhoeas* and *P. laevigatum* which is of the *Rhoeas* type. A group of F_2 's consisted of 32 red plants with a black blotch like the *laevigatum* parent, nine rose pink with a white blotch like the *Rhoeas* parent and one light red with no blotch. From this result he concluded, as also did Kajanus (1919) from an F_1 between two *Rhoeas* forms, that the black blotch was controlled by a factor which was epistatic to a factor producing a white blotch. Both factors in the recessive condition produced the absence of a blotch. This plant with no blotch apparently was not tested, and in view of its colour and the fact that the production of the black blotch is variable it can safely be included in the red class with black blotches. This gives 33 black : 9 white blotch, clearly a 3 : 1 ratio.

Rasmuson also found that white latex was recessive to yellow. Only two families totalling 12 plants segregated for latex colour, and he concluded that yellow and white differed by a single factor. These numbers, however, are too small to come to such a conclusion, and, as the present experiments show, latex colour is controlled by more than one factor.

Negodi (1932) studied the F_1 and F_2 of a cross between *P. Rhoeas* and a white-flowered form. The F_1 consisted of plants with flowers like *P. Rhoeas*, and all except one had a white petal edge. The F_2 showed a range of types like those found by the present author in a similar F_2 . No evidence of tests of the constitution of these F_2 types is given, yet Negodi groups F_2 classes in such a way that he explains his results on a two-factor basis where one of the factors for flower colour in the recessive condition is also supposed to produce a white petal edge. He also applies this hypothesis to the results of Béguinot (1928). This hypothesis, of course, is simple but erroneous, and there is no doubt that the results can be explained on the same basis as given here. The F_1 was clearly heterozygous for at least four factors **c**, **b**, **W** and **t**.

The variation in the amount of crossing-over between individual families shown in Table III on the whole cannot be regarded as significant, and the massed results, particularly of back-crosses, probably give a close approximation to the real value. The following points are of interest. First, the eight factors for flower colour and the factor for hair colour fall into three (or perhaps two) linkage groups, although there is a possibility of seven linkage groups. Secondly, on the basis of three

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linkage groups the factors are all very tightly linked with the exception of **p**. Thirdly, two of these factors have presumably mutated to a dominant condition over the wild type.

These facts may or may not be significant, but they bear a resemblance to the results obtained in *Paratettix texanus*, where 25 or more dominant factors over the normal for colour pattern are extremely closely linked on one chromosome and only two or three other factors are apparently carried by other autosomes. Other extreme cases of this type are those of *Apotettix eurycephalus*, where 13 factors for colour pattern and a lethal factor are closely linked on one autosome (Nabours, Larson and Hartwig, 1933), and of *Lebistes*, where 17 out of 18 dominant factors for colour are carried by the sex-chromosomes (Winge, 1927).

It is possible that the effect of natural selection upon the evolution of *P. Rhoeas* and the above examples has been similar, and with regard to these factors which have undergone mutation, their concentration among and along the chromosomes may have given the present-day species a biological advantage over its ancestors.

SUMMARY.

Wild *Papaver Rhoeas* has been found to be of the following constitution for eight flower-colour factors: **CCPPBBTTwwffIIIEE**. The phenotype for various combinations of these factors and their allelomorphs is given and the effects and interaction of the factors is described.

The factors fall into three linkage groups, (1) **bW**, (2) **pFit**, (3) **ec**. The locus of **t** is uncertain, but it is close to **F** and **i**. There are probably two factors for hair colour, and one of these is in the first linkage group and lies on the left of **b**, *i.e.* the order is **sbW**.

There is no difference in segregation or crossing-over for these factors between the male and female sides.

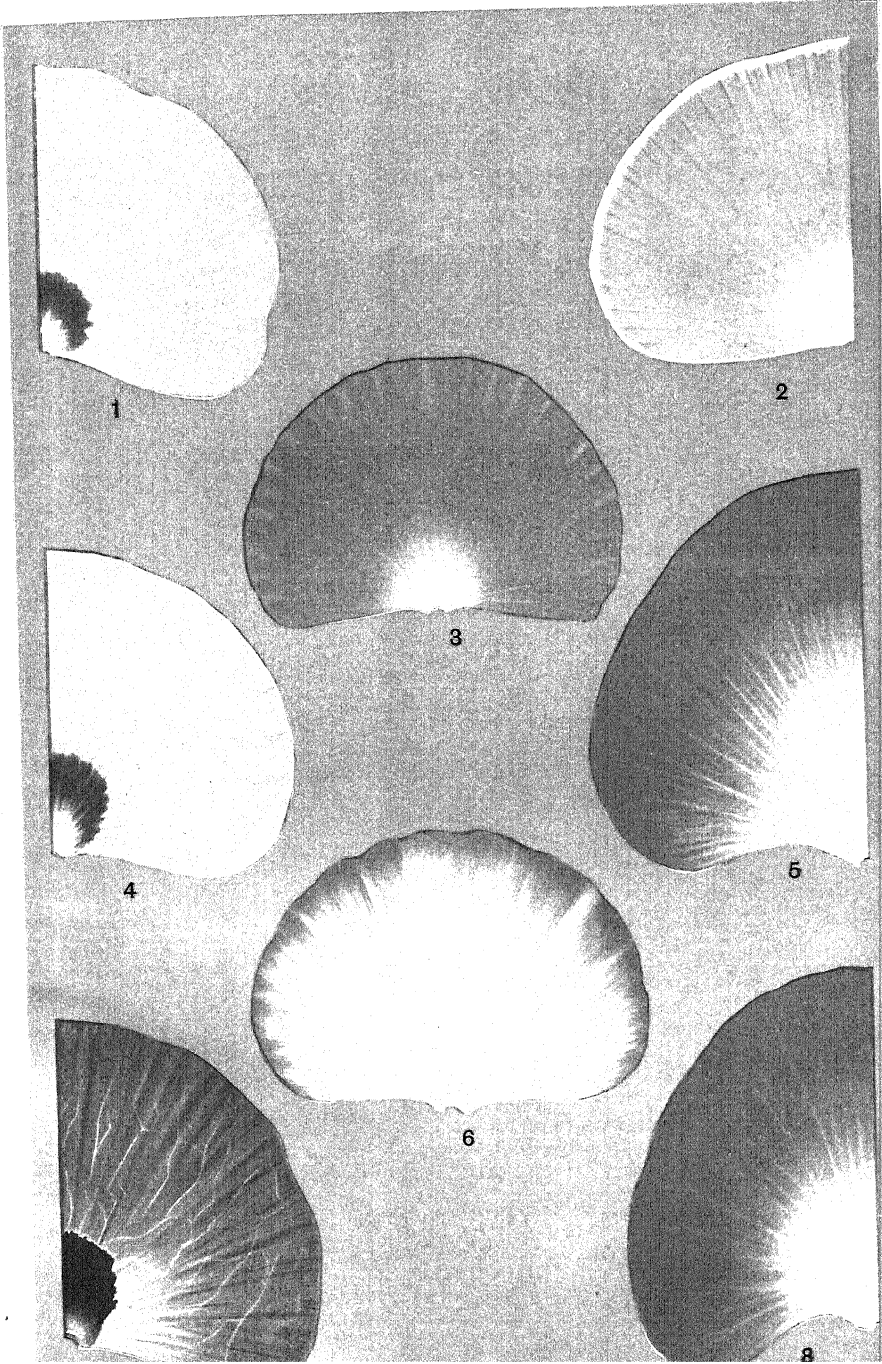
Another factor **m** for flower colour (no white margin to the black blotch) is shown to be independent of **p**.

Doubleness is probably incompletely recessive to singleness, and its complicated genetical behaviour indicates that it is controlled by several factors.

Albinism is recessive and differs from the normal by a single factor, which is independent of the factors for flower colour.

Latex colour is probably controlled by two factors and is affected by the factor **p**.

Characters of the foliage and habit which do not lend themselves to accurate study are mentioned.



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EXPLANATION OF PLATE VIII.

From paintings kindly done by Mr H. C. Osterstock.

- Fig. 1. Salmon pink netted white, brown blotch with white margin. The colour is more yellowish than is shown. **ceti**.
- Fig. 2. Salmon, white blotch, white edge to petal. The colour is more yellowish than is shown. **cbet**.
- Fig. 3. Rosy scarlet, white blotch. **btF**.
- Fig. 4. Lilac, brown blotch with white margin. **cpeti**.
- Fig. 5. Fiery red, flushed white centre, white blotch. **cbetiF**.
- Fig. 6. Cream edged rose crimson, white blotch. **cbtiF**.
- Fig. 7. Crimson or cherry, netted and flushed white, black blotch with faint white margin **cti**.
- Fig. 8. Dull carmine lake, flushed white centre, white blotch. **cpbetiF**.

INTERSPECIFIC AND INTERGENERIC HYBRIDS IN HERBAGE GRASSES. INITIAL CROSSES.

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INTRODUCTION.

IN his "Apology" to the work entitled *Gramina Pascua*, published in 1790, Swayne wrote as follows: "A difference in soil and situation will often occasion so striking a difference in the appearance of these Plants, as has, no doubt, been the cause of determining many to be distinct

species, which in reality are only varieties. And perhaps an adventitious admixture of the *Pollen* from different species may often produce hybrid plants, which might not uncommonly have deceived the Investigator. Nor is it to be wondered at, if this should *very frequently* happen amongst plants which are so nearly allied, whose generating organs are apparently so similar, and which are situated so close together as the Grasses generally are. It is rather wonderful, considering the circumstances, that the real species of Grasses should have continued so distinct as we find them; when it is well known what infinite and dissimilar varieties have been produced by such an admixture in several other species of plants."

At the time when Swayne wrote the paragraph from which this quotation has been taken, he considered *Festuca loliacea* to be a variety of *F. pratensis*, but it is evident that even at this time he was quite prepared to adopt a different view, provided he found sufficient reason for doing so, although he does not refer to this plant in particular. He was also obviously in a mood to pay attention to the subject of grass hybrids, with the result that by the time Withering's third edition of *An Arrangement of British Plants* appeared in 1796, he had suggested to the latter author that the plant should be known as *F. hybrida*, since he was now convinced that it had originated from "an intercourse between" *F. elatior* var. *pratensis* and *Lolium perenne*.

As far as I have been able to ascertain, no suspicion that *F. loliacea* might be a hybrid, nor that any natural hybrids between any two species of the herbage grasses existed, had previously been made, and even as recently as 1882, Hackel in his monograph on the fescues, following Ascherson (1864) and Focke (1881), states that the theory that *F. loliacea* is a hybrid of the type described originated with Braun (1834). It is therefore necessary to examine Swayne's position somewhat more closely. He came to the conclusion that it is a hybrid "from its constant infertility," and that its origin is as described above, "from its resemblance to both *F. elatior* var. *pratensis* and to *L. perenne*."

It is clear that in Swayne's mind "infertility" referred only to failure to set seed, since in the same letter, quoted by Withering, he states that "the stamens were apparently perfect, and shed a large quantity of pollen..." This statement may give rise to some doubt whether, in fact, Swayne was referring to true *F. loliacea*, since it is generally agreed that this plant does not liberate pollen (see e.g. Schultz, 1854, 1857). Before coming to this conclusion, however, it should be observed that Swayne had transferred "some roots" into his garden and later increased this

material by vegetative propagation "till it occupied a plot six feet square." As pointed out elsewhere (Jenkin, 1931 *a*), great care is required in work of this kind on account of the fact that in a dense sward the tillers of different plants become closely interwoven, so that it is quite possible that amongst the "roots" which Swayne originally transferred into his garden there was a single rooting of *L. perenne* which would not be easily distinguishable from true *F. loliacea*. The subsequent increase of this garden material would automatically include the increase of the stray *L. perenne* plant, so that in the 6 ft. square plot it would be considerably represented and would produce "much pollen." If, then, only a single rooting of *L. perenne* had been taken in the first instance, the absence of seed is easily explained on the basis that *L. perenne* plants are often very highly self-sterile (Jenkin, 1931 *b*).

In very much the same way it may be argued that Hudson was misled by impure material to the extent that, while at first (1762) he regarded *F. loliacea* as a distinct species, he later (1778) came to regard it as merely a variety of *F.* (= *Glyceria*) *fluitans*.

Curtis (1777) had observed the great similarity of plants classifiable as *F. loliacea* to *F. pratensis* on the one hand and to *L. perenne* on the other, but neither he nor Hudson expressed any suspicion that they were hybrids.

Swayne was obviously puzzled by Hudson's position, and states with regard to his own material, "It should seem to be the *Festuca loliacea* of Hudson but that I am confident it never originated from the seeds of *F. fluitans*." It is possible, however, that the association of *F. loliacea* with *F. fluitans* led to the position that is described by Sinclair (1816), when he states that "some Botanists have supposed it to be a hybrid, the joint produce of the *Lolium perenne* (rye-grass) and *Festuca fluitans* (flore fescue)." Sinclair himself, however, argued in favour of *F. pratensis* as one of the parents, with *L. perenne*, and in the interval, Knapp (1804) had expressed an opinion similar to that of Swayne.

There is no reason to believe that either Braun (1834) or those continental writers who later supported him (Focke, 1864; Ascherson, 1864)¹ were aware that in this country the theory of hybrid origin was well known many years previously. This position is perhaps not to be wondered at, since I have failed to find any reference to the theory in the later writings of Curtis, while Smith (1828) fails to mention it, although he refers specifically to the particular edition of Withering referred to above and also to Knapp and Sinclair.

¹ Crépín (1863) accepts the theory but does not refer to its origin.

Similarly, on the Continent, Ascherson (1864) states that the view expressed by Braun was at first either ridiculed or ignored by systematic botanists, and this is exemplified by Koch (1857) who regarded *F. loliacea* as a good species and does not refer to the hybrid origin theory.

It is possible that there still remain those who consider the plant to be either a distinct species or a variety of *F. pratensis* (see Ward, 1908; Armstrong, 1917), but the general thesis of natural hybridisation in the herbage grasses has now gained very wide recognition. Indeed, it is possible that in recent years the tendency has been to classify unusual types too readily as hybrids.

The characters upon which these grasses are classified as hybrids by taxonomists are mainly the following: (1) morphological characters, (2) sterility, (3) the association of the supposed hybrids with their reputed parents in a particular habitat. Evidence based upon these considerations is undoubtedly important, but at least two things may be borne in mind: (i) Even within a definite species the variation in morphological characters is often extremely wide. This fact is most strikingly illustrated, for instance, when a very large and representative population of such a species as *L. perenne* is brought together and studied as single plants. (ii) Male-sterile plants (which may be fully female fertile) have been found within several different grass species (Jenkin, 1931 *a*, p. 25; 1931 *b*).

While it may be readily admitted that evidence of the type mentioned is very valuable, it is too much to say that in any particular case it can be regarded as amounting to absolute proof. Perhaps such absolute proof cannot be produced, but it is evident that further valuable information can be obtained by means of other methods. One of these, the cytological investigation of the supposed hybrids and their reputed parent species, has been applied by Levan (1930) in the case of the supposed hybrid between *Dactylis glomerata* and *D. Aschersoniana* and by Huskins (1930) to *Spartina Townsendii*.

Another method, the one adopted in the experimental work at present to be described, is the study of the compatibility of the supposed parent species, and, if F_1 and other generation hybrids can be produced under such controlled conditions that mistakes and mishaps are practically impossible, to compare the artificially produced plants with their supposed naturally produced counterparts.

Work which might have been of very considerable interest and value in connection with some of the combinations here to be described was initiated, according to M'Alpine (1898), by Garton about 1893. It is,

however, perfectly clear that Garton was not in any way interested in the origin of natural hybrids and that his main idea in attempting to make interspecific and intergeneric crosses was to induce "sporting." M'Alpine obviously shared the same view, with the result that the account of this work is of very little use in the present connection. In fact, it is quite impossible to know exactly what happened. Illustrations of inflorescences are given, but they are stated to represent "the second progeny of the cross"—a statement which obscures rather than elucidates the position. The illustrations themselves certainly suggest that some true hybrids were produced, while M'Alpine, an agrostologist of the highest repute, states in the case of "Meadow Fescue on Perennial Ryegrass": "there is absolutely no dubiety here; the perennial ryegrass and meadow fescue have become blended and mixed in one and the same plant." He attempts to justify this statement by means of descriptions of the hybrids, but this evidence, as given, is far less convincing than the fact that the assertion is made by M'Alpine, since it is based on the presence together in the same plant of two types of shoot. These two types may actually occur in pure *L. perenne* at a particular growth stage. Combining M'Alpine's assertion with the illustrations given, however, there remains no room for reasonable doubt that Garton did, in fact, succeed in producing intergeneric hybrids.

When, in 1921, I first had the opportunity to give practical attention to interspecific and intergeneric hybrids in the herbage grasses, I was unaware of Garton's work, but was conscious of the lack of proof with regard to the hybrid origin of *F. loliacea*. In that season, however, no plants of *F. pratensis* were available, and, therefore, I decided to attempt certain other crosses, which, as will be shown below, gave interesting results. In the following year, 1922, *F. pratensis* plants were available and certain crosses were made. In still later years the work has again been extended, as will be seen from Table I, which gives a summary of the initial results obtained. In the present paper it is not intended to take the account of the work beyond the point of whether established plants were obtained together with a brief statement as to whether such plants were or were not male fertile.

METHODS AND DEFINITIONS.

The methods employed in the artificial hybridisation of grasses have been described in detail elsewhere (Jenkin, 1924, 1931 *a*)¹. It is, therefore, only necessary here to give a few particulars.

¹ I may here also refer to the method used by Nafziger (1918) in the hybridisation of *Sorghum*. I was until quite recently unaware of the existence of the paper referred to.

All the work to be described has been carried out under cool greenhouse conditions. The parent plants are grown in pots and are brought in well in advance of flowering and remain indoors until the seed is harvested. The greenhouses are thrown open during the day in order that the temperature may be kept as normal as possible, but in the evenings they have perforce to be closed down for pollination purposes, and then the temperature is apt to rise far above the normal. Overnight, the lights are left open, but there is no free circulation of air, and both during pollination and during the night the atmosphere becomes very humid.

Female units, consisting of a varying number of emasculated florets, are prepared and bagged up well in advance of flowering. These are later pollinated daily, as the stigmas appear, provided pollen is available.

The method, and the conditions under which it is applied, are capable of giving practically perfect seed setting, caryopsis development, seed germination and plant establishment in intraspecific crosses. In such crosses, the limits of success are determined by incidental conditions, particularly those affecting flowering and pollen liberation. Absolutely perfect pollinating conditions have rarely been obtained in the present work, so that in comparing seed setting in reciprocal crosses or in different crosses a wide margin of error must be allowed.

The term "heavy seed" as used in the present connection is an elastic one and includes all florets in which the ovary appears to have been in any degree stimulated, irrespective of the measure of caryopsis development that has resulted.

Representatives of a few lots of seed have been planted directly in sterile soil in order that the method might be tested, but since by this method it is impossible to say whether very weak attempts at germination have been made, laboratory incubation has been almost exclusively used. The seeds are placed on a filter-paper pad on the Copenhagen type of incubator and are kept under constant observation, the germinating seeds being pricked out into sterile soil.

The aggregate results are shown in the table below, but in an aggregation of this kind certain important points are obscured. These are dealt with in the text.

RESULTS OBTAINED FROM VARIOUS COMBINATIONS.

(See Table I.)

Cross I: *Lolium perenne* + var. with *Festuca pratensis*.

The references to the supposed natural hybrid, *F. loliacea* Curt., are very numerous in the literature and need not be dealt with in detail.

TABLE I.

Showing aggregate results for the initial interspecific and intergeneric crosses.

Cross		Gametic type of cross**	Seasons when crosses were made	No. of florets enaculated	No. of heavy seeds	Percent- age seed setting	No. of seeds incubated	No. of germinating seeds	Percent- age germination	No. of plants established	Remarks
♀	♂										
<i>Liatium (perenne)*</i> × <i>Festuca pratensis</i>		7 + 7	1922, 1928-31	2046	841	41.1	841	6	0.7	1	
<i>F. pratensis</i> × <i>L. (perenne)</i>		7 + 7	1922, 1928-31	1513	408	27.0	408	20	4.9	7	
<i>L. tumulentum</i> × <i>L. perenne</i>		7 + 7	1922	42	21	50.0	21	1	4.8	1	
<i>L. perenne</i> × <i>F. ovina</i>		7 + 14	1930	150	25	16.7	25	0	—	—	Seeding not a true hybrid.
<i>Glyceria fluitans</i> × <i>L. perenne</i>		14 + 7	1931	182	0	—	—	—	—	—	Pollination conditions poor.
<i>L. perenne</i> × <i>Dactylis glomerata</i>		7 + 14	1921	16	0	—	—	—	—	—	Well pollinated.
<i>L. perenne</i> × <i>Arrhenatherum avenaceum</i>		7 + 14	1921	12	0	—	—	—	—	—	
<i>L. perenne</i> × <i>F. arundinacea</i>		7 + 21	1921, 1928, 1929, 1931	654	215	32.9	205	81	39.5	75	
<i>F. arundinacea</i> × <i>L. perenne</i>		21 + 7	1921, 1928-30	429	115	26.8	112	0	—	—	
<i>F. pratensis</i> × <i>F. arundinacea</i>		7 + 21	1924, 1928	235	136	53.6	126	40	31.7	24	
<i>F. arundinacea</i> × <i>F. pratensis</i>		21 + 7	1924, 1928	173	86	49.7	86	27	32.6	20	
<i>F. pratensis</i> × <i>F. gigantea</i>		7 + 21	1930	46	28	60.9	28	8	34.8	6	Estimated total germination = 42.9 %.
<i>L. perenne</i> × <i>F. rubra</i>		7 + 21	1921, 1924, 1928	218	99	45.4	77	14	18.2	9	
<i>F. rubra</i> × <i>L. perenne</i>		21 (or 26) + 7	1921, 1922, 1928	181	13	7.2	13	0	—	—	
<i>F. pratensis</i> × <i>F. rubra</i>		7 + 21 (or 28)	1922, 1928, 1930, 1931	679	72	10.6	72	0	—	—	
<i>F. rubra</i> × <i>F. pratensis</i>		21 (or 28) + 7	1928, 1930, 1931	432	53	12.3	52	0	—	—	
<i>F. ovina</i> × <i>F. rubra</i>		14 + 21 (or 28)	1930	223	7	3.1	7	3	42.9	2	Pollination very incomplete.
<i>F. rubra</i> × <i>F. ovina</i>		21 (or 28) + 14	1930	132	3	2.3	3	0	—	—	Pollination very incomplete.
<i>F. arundinacea</i> × <i>F. rubra</i>		21 + 21	1921, 1928	273	22	8.2	22	0	—	—	
<i>F. rubra</i> × <i>F. arundinacea</i>		21 + 21	1921, 1928	224	91	40.6	91	7	7.7	7	
<i>F. gigantea</i> × <i>F. arundinacea</i>		21 + 21	1930	104	77	74.0	57	51	89.5	51	Estimated total germination = 92.2 %.
<i>F. arundinacea</i> × <i>F. gigantea</i>		21 + 21	1930	60	7	11.7	7	7	100.0	7	Pollination very incomplete.

text for types of plants actually used.

e authorities for the chromosome numbers of the various species are given in the text, except the following: *Liatium tumulentum*, somatic number = 14, Faworski (1927); *Glyceria fluitans*, number = 28, Stählin (1929); *Dactylis glomerata*, Davies (1927), Stählin (1929), Kattermann (1930), Levan (1930); *Arrhenatherum avenaceum*, Aase and Powers (1926).

Curtis (1777) observed that within *F. loliacea* great variation in inflorescence type may be found, and with regard to this there appears to be very general agreement. Such variations led Crépin (1863) to abstain from quoting any references, since he believed that the name covers two different forms even apart from those which are more or less awned. An awned type, according to the literature, was designated *F. Braunii* by Richter in 1890. This had previously been known as *F. loliacea* var. *aristata* A. Br., and was supposed to be a natural hybrid between *F. pratensis* and *L. italicum* A. Br. (= *L. multiflorum* Lam. = *L. perenne* var. *multiflorum*). As early as 1856, according to Holmberg (1930), this interpretation had been questioned, and doubts were also expressed by Ascherson and Graebner (1900-2). Holmberg himself (*loc. cit.*) considers that all specimens previously described as hybrids of this type were in fact hybrids between *L. perenne* and an awned, or at least mucronate, type of *F. pratensis*, but he claims to have discovered a plant which is unmistakably a natural hybrid between *F. pratensis* and *L. multiflorum*.

Thus, in nature, there are supposed to exist awnless forms which may approach *F. pratensis* on the one hand, or *L. perenne* on the other, while there are also two more or less awned types of different origin.

Taxonomists therefore claim that either of the two forms of *Lolium* may interbreed successfully with *F. pratensis*. This fact is of some little importance in connection with the present crosses, since two of the plants actually used have resembled the variety *multiflorum* much more closely than typical *L. perenne* plants. One of these, plant bX-3, is of unknown origin, and may be a pure *multiflorum*, although actually it was not quite typical for this form. It happens to be of relatively little importance, however, since it left no progeny. The other plant, 21-bE-1, on the other hand, is of great importance, since it is the only *Lolium* plant that has, in these experiments, given rise to hybrids with *F. pratensis* when the latter was used as the female parent. Moreover, the results obtained in that particular case were surprisingly high. It is therefore necessary to give a brief description of this plant. Its female parent, plant bA-10, is known to be a typical *L. perenne* plant. This has consistently given progeny, either when self-pollinated or when used as a parent in intra-type crosses, which are also typical *L. perenne*. Plant 21-bE-1, on the other hand, resembles the *multiflorum* type rather closely. This is usually the case when *L. perenne* and its var. *multiflorum* are intercrossed, so that it appears safe to assume that plant 21-bE-1 is a hybrid of this type.

This raises the question whether a plant of this type is, in virtue of

its origin, likely to give results when it is intercrossed with *F. pratensis*, which are different from those obtainable when typical *L. perenne* plants are similarly used.

It has been shown elsewhere (Jenkin, 1931 c) that *L. perenne* and *L. perenne* var. *multiflorum* intercross quite readily¹ and give progeny which are similar with regard to fertility characteristics to plants derived from intraspecific crosses. Evans (1926) also found that the divisions in the pollen mother cells of such artificially produced hybrids are regular except that lagging chromosomes are occasionally seen. Peto (1933) states that in plant 21-bE-1, a very considerable proportion of the pollen is bad, but it should be explained that since the plant is very similar to the *multiflorum* type it is only kept alive by vegetative propagation with considerable difficulty, and the plantlets examined by Peto were decidedly lacking in vigour. On the other hand, since the plant was used for the present crosses as the male parent, there was no serious lack of pollen when the plant was at the height of its vigour.

On the whole, therefore, it seems improbable that, in virtue of its origin, plant 21-bE-1 should be expected to give results differing essentially from those obtainable from the use of typical *L. perenne* except in so far as its genetical constitution might affect the morphology and physiology of its progeny.

Cross I a. *Lolium perenne* + var. ♀ × *Festuca pratensis* ♂. A number of crosses of this type were made in 1922, using both typical *L. perenne* plants and the two non-typical plants referred to above. Only one small unit of 40 florets was prepared on plant bX-3, but it yielded 25 "heavy seeds," none of which germinated. Several similar units were prepared on plant 21-bE-1, and a seed setting of 24.6 per cent. resulted. None of these seeds germinated, so that, used as female parents with *F. pratensis* as the pollen parent, no germinable seed was obtained from either of these two non-typical plants.

In the same season, several female units were prepared on true *L. perenne* plants, but only 29 "heavy seeds" were obtained from 273 florets (= 10.6 per cent.). Five of these, however, all from a cross between the *L. perenne* plant bA-67 and *F. pratensis* bF-3, germinated, but the seedlings were extremely weak and only one survived to become an established plant (= plant 30-bE-1: see Peto, 1933). This plant, although capable of surviving the early growth stages only as the result of careful nursing, has become a moderately vigorous plant and can be

¹ Nilsson (1930 b) has reached a different conclusion, but the significance of his results has been questioned (Jenkin, 1931 c).

propagated vegetatively without serious difficulty. It is functionally male sterile.

Many further attempts to intercross true *L. perenne* plants and *F. pratensis*, using the former as pistillate parents, have been made in recent years, but out of 726 heavy seeds obtained in the seasons 1928-31, only one has shown any signs of germination. Persistent efforts failed to keep this seedling alive, but it may be a significant fact that this seedling was also produced by the pair of plants successfully used in 1922. This suggests that these plants, while giving very poor results, are more compatible than the other pairs used.

So far, therefore, only a single established plant has yet been obtained from 2046 *L. perenne* + var. florets emasculated¹.

Cross I b. *Festuca pratensis* ♀ × *Lolium perenne* + var. ♂. On the average, seed setting was lower when the cross was made in this direction, but germination was somewhat higher.

In 1922, no typical *L. perenne* plants were available when the female units prepared on *F. pratensis* plants were ready for pollination. Consequently the two non-typical plants bX-3 and 21-bE-1 were exclusively used in that season. The former yielded 14 heavy seeds from 69 florets, but none of them germinated. When the pollen of plant 21-bE-1 was used, 41 heavy seeds were obtained from 62 *F. pratensis* florets (= 66 per cent.). Eighteen of these (= 44 per cent.) germinated, but, as in the direct cross, germination was extremely weak, and only seven seedlings survived to become established plants, and four of them have since shown considerable constitutional weakness. All those that have reached the flowering stage are functionally male sterile.

In later seasons typical *L. perenne* plants have been used as pollen parents, but only two germinable seeds have been obtained from 1382 *F. pratensis* florets emasculated and pollinated as compared with a single germinable seed from 1482 florets when the cross was made in the opposite direction in the same seasons, so that apart from season 1922, the yield of germinable seed has been extremely low in both cases, and in neither case did the seedlings survive.

If we include the results for 1922 in a general average (as shown in Table I), we find that seed setting was higher when *Lolium* was used as

¹ In 1932, similar crosses were again made, but the *L. perenne* plant bA-67 was not used. From 822 florets emasculated, 228 heavy seeds (= 27.7 per cent.) were obtained. Three of these (= 1.3 per cent.) have germinated, but it is yet too early to know whether any of them will survive. At the moment, it seems probable that two of them will become established plants. The *L. perenne* plant which gave rise to these seeds had not previously been used for this purpose.

the female parent. The margin of difference, however, is not particularly wide, and it is possible that the figures as given err on the high side in Cross I a, owing to the fact that when *Lolium* is used as the female parent in this particular combination it is difficult, even with the aid of a diaphanoscope, to determine with certainty whether in some cases the ovary shows signs of stimulation or not. It is therefore possible that some florets have been classed as "heavy" when they were actually "light." The same difficulty is never found when *F. pratensis* is used as the female parent, since the "heavy" and "light" can easily be distinguished. In fact, although the two parent species are similar in chromosome numbers, caryopsis development is strikingly dissimilar according to whether the one or the other is the pistillate parent. When *Lolium* is used in this capacity the caryopsis rarely reaches beyond half the length of the paleae, but at its best development it is then plump in relation to its length, hard and smooth. From this extreme development every possible grade down to the point where it is impossible with certainty to determine whether the ovary has been stimulated may be found, but in practically all cases the caryopsis is plump in relation to its length. When *F. pratensis* functions as the female parent, on the other hand, the caryopses rarely fail to reach half the length of the paleae, and at their extreme development they are even slightly longer than the paleae and therefore also longer than those normally produced by the species. In all cases, however, they are slender, very badly shrivelled, and not infrequently deformed, particularly at the tip. Yet, with the exception of the single case where a non-typical *Lolium* plant was used as the male parent, there appears to be no material difference in germinating capacity.

Since all the seeds available each season are used for germination tests in the hope that additional established hybrids may be obtained, it has not been possible to examine germinable seeds except by observation. Those which have failed to germinate have, however, been examined by dissection under a binocular dissecting microscope of high power. But since the seeds are allowed about three weeks on the incubator, it is possible that important changes have taken place. Yet, since these seeds differ according to the direction of the cross, the observations made are of interest. With *L. perenne* as the female parent, the better seeds which have failed to germinate contain perfectly plump and smooth caryopses. When dissected, these show no signs of degeneration¹ and release no watery fluid when pricked. There is a well-formed and apparently well-

¹ Germination usually takes place in six to eight days, so that after three weeks on the incubator it is safe to assume that no further germination will occur.

organised endosperm which is firm but not hard. So far it has not been possible to detect the presence of an embryo¹.

The caryopses derived from *F. pratensis* on the other hand, although they have certainly swollen, are yet not fully distended and remain more or less wrinkled. When pricked, they release a watery fluid, while no trace of an organised endosperm can be found on dissection. At most, the inside is more or less lined with a blackish pulp which is obviously degenerating, and despite their relatively great size the caryopses are practically empty. No trace of an embryo has been found in such seeds.

Neither of these two classes of caryopses can fully represent those seeds which have succeeded in showing signs of germination, but they probably indicate the general position with regard to endosperm development. Whatever may have happened in the early stages of caryopsis development, it is safe to say that in the very great majority of the seeds the ultimate result is very different according to the direction of the cross. As far as a sufficiency of endosperm is concerned, some of the *L. perenne* ♀ × *F. pratensis* ♂ seeds which fail to show any signs of life on the incubator should be capable of germination, so that very probably in these cases the embryo is at fault. In the seeds from the reciprocal cross it is probable that both endosperm and embryo are usually defective, but occasionally, as shown by the fact that some germinable seeds have been obtained, the embryo survives. The lack of endosperm would in this case be sufficient to account for the extreme weakness of the seedling, but in the opposite cross it is probable that the embryo finds it difficult to make use of the endosperm available. However, it has been impossible to determine what stage of development the endosperm has actually reached in the germinating seeds, and it is conceivable that the embryos which develop and persist are not those of the plumpest seeds.

Descriptively, at least, these two types of caryopses very closely resemble those obtained by Watkins (1927 and elsewhere) from the inter-specific cross *Triticum turgidum* with *T. vulgare*, but while in the present case the cross is between diploids the wheat crosses were of the type tetraploid with hexaploid. The similarity perhaps is essentially one of descriptive characters, since, as far as the present meagre data are capable of showing, the smaller caryopses do not germinate better than the others. In any case, the apparent parallelism is of very considerable interest.

On the available evidence the type of caryopsis produced is similar whether typical *L. perenne* plants or the non-typical *Lolium* plant 21-bE-1

¹ Quite possibly the method of examination is inadequate for this purpose.

be used, but the latter, when used as the pollen parent, gave seed of exceptionally high germinating capacity. It has been argued above that a difference in the results should not be expected as a direct result of the antecedents of plant 21-bE-1, and yet at first sight the results appear to suggest that plant 21-bE-1 has given results which are essentially different from those given by typical *L. perenne* plants. Before this conclusion should be reached, however, there are various considerations to be borne in mind. (1) Plant 21-bE-1 when used as the female parent in the same season failed to give germinable seed. (2) Plant bX-3, which was very similar morphologically to plant 21-bE-1, failed to give germinable seed in the same season, either when used as the male or as the female parent. (3) In the direct cross with *Lolium* as the female parent a greater proportion of germinable seed was obtained in 1922 than in any subsequent season. (4) An established plant was obtained from seed resulting from the cross *L. perenne* ♀ × *F. pratensis* ♂ in the same season, but not subsequently up to and including 1931. Presumably, therefore, 1922 was a particularly favourable year. (5) The only germinable seed from the cross *L. perenne* ♀ × *F. pratensis* ♂ obtained subsequently (again up to 1931) was of exactly the same parentage as those obtained in 1922. This suggests that this particular combination, *L. perenne* bA-67 with *F. pratensis* bF-3, shows an unusual degree of compatibility. It might, therefore, be argued that the high results obtained by the use of plant 21-bE-1 in 1922 may be partly due to the season and partly to a high degree of compatibility with the *F. pratensis* plant bF-2, but this high degree of compatibility is not of necessity a characteristic which is due to its being derived, presumably, from a cross between *L. perenne* and its variety *multiflorum*. In fact, it is well known that success in interspecific or intergeneric breeding may depend to a very great extent upon the selection of the strains or varieties to be employed in such crosses (Backhouse, 1916; Thompson, 1926; Meister and Tjumjakoff, 1928; Kostoff, 1930; Hollingshead, 1932).

The extremely low germinating capacity of the seeds and the extreme weakness of the seedlings obtained when the cross is made in either direction is remarkable for two reasons. (1) No such serious difficulties have been encountered in certain crosses which, from differences in the morphology of the parent species and in chromosome numbers, might be expected to cross less easily. In particular, the contrast between *L. perenne* ♀ × *F. pratensis* ♂ and *L. perenne* ♀ × *F. arundinacea* ♂ (see below) may be noted. (2) In nature, the supposed hybrid between *F. pratensis* and *L. perenne* (= *F. loliacea* Curt.), judging by the taxonomic

records, has a far wider distribution than any of the other hybrids discussed in the present paper. Moreover, although Braun (1834) found that the plant occurs only in small groups, Schultz states that it covers wide stretches of ground around Wiessenburg, while Holmberg (1930) refers to an extensive sward of *F. loliacea* near Landskrona. Its distribution in this country has not been worked out in detail, but Curtis (1777) found it to be abundant in the Battersea meadows, while in an area near Oxford, which I examined in 1931, I found plants broadly classifiable as *F. loliacea* to be unusually prevalent. Supposing, then, that all these plants, classifiable as *F. loliacea*, are in fact naturally produced F_1 hybrids between *F. pratensis* and *L. perenne*, it might be expected that such hybrids could be produced with relative ease under artificial conditions, particularly since hybrids have been produced between species which do not appear to intercross very freely in nature.

But before concluding that the present results are in any way fatal to the supposition that *F. pratensis* and *L. perenne* under field conditions are capable of giving rise to established F_1 hybrids, there are various points that must be taken into consideration. (1) The paucity of other hybrids in nature may be mainly due to factors other than the incapacity of the plant pairs to intercross successfully. In particular, a difference in habitat requirements or in time of flowering might alone be a sufficient reason why the records are so meagre. In addition, some of these hybrids are less strikingly different from one or the other parent, and might easily be overlooked. (2) The artificial conditions may be less favourable for the cross between *F. pratensis* and *L. perenne* than to some of these other crosses. (3) The conditions in the field, at least in certain seasons or in certain localities, may be definitely more favourable than those of the greenhouse in respect of caryopsis development, seed germination and plant establishment¹. The artificial conditions involve (a) bringing the plants into a cool greenhouse before they come into flower and keeping them there until the seeds have been harvested, (b) an abnormally high atmospheric humidity at night, (c) abnormally high temperatures at certain times, (d) manipulation with some mutilation of the inflorescences, (e) absence of pollination immediately after stigma exertion, since, while the flowers open usually before noon, the pollinations are made, for reasons explained elsewhere (Jenkin, 1924, 1931 a), late in the evening, and (f) the amount and quality of the light reaching the inflorescences is affected by the glass and by the isolating paper bags. As already pointed

¹ Cf. Collins and Mann (1923).

out, however, intraspecific crosses and even some interspecific and intergeneric crosses succeed quite well under these conditions, so that the present crosses must be particularly sensitive if the poor results are entirely due to the conditions. On the other hand, none of these conditions exists in the field, and in addition, it may be pointed out that quite possibly in those localities where *F. loliacea* occurs in any considerable quantity the average climatic conditions are more favourable than those of the Aberystwyth district, even apart from the artificial modifications here necessarily introduced.

On the other hand, in at least one respect the artificial conditions may be expected to be more favourable than those of the field. If, in a particular area, the two parent species occur in approximately equal quantities, both intraspecific and interspecific pollination would take place, and the success of the "foreign" pollen would depend upon its capacity to compete with pollen of the same species as the mother plant involved. Cases are known where the "foreign" pollen is the more effective (Kostoff, 1930) or equally effective (Kihara, 1932, quoted by Kihara and Nishiyama, 1932), so that in the present case the expectation may not be justified. Moreover, circumstances may arise where pollination is, at least in the case of some stigmas, entirely from the "foreign" species. Thus, where a single highly self-sterile plant of *F. pratensis* occurs in a population of *L. perenne*, apart from the plant's own pollen (assumed to be highly ineffective), its only chance of setting seed would be from interspecific pollination. If such *F. pratensis* plants were widely scattered, there would still be the chance that pollination would be mainly from *L. perenne*. Further, plants definitely classifiable as *L. perenne*, but male sterile, are apparently always female fertile (Jenkin, 1931 b). If such plants are particularly prevalent in an area, there will obviously be a deficiency of *L. perenne* pollen, and again more or less pure pollination from *F. pratensis* might take place on many *L. perenne* stigmas. This latter case is not purely hypothetical, since in the Oxford area already referred to such male sterile *L. perenne* plants appeared to be unusually prevalent¹.

The present results, therefore, poor though they are, do not prove that F_1 hybrids between *F. pratensis* and *L. perenne* cannot be produced in nature, although the seedlings actually produced in the present ex-

¹ The question may arise later whether these were in fact pure *L. perenne* although classifiable as such, or whether at least to some extent they were the later derivatives of the hybrids. There is good reason to believe, however, that male sterile plants occur which are not such in virtue of interspecific crossing, so that the argument still stands.

periments would apparently have not the slightest chance to succeed in competition with seedlings produced intraspecifically.

There still remains the fact that *F. loliacea* is particularly well represented in certain areas. It is possible that the conditions are so far superior in those areas, as compared with those of the present experiments, that the hybrids are actually produced in great abundance, but it is not essential that this should be the case. Established plants of *F. loliacea* have attracted attention in some cases (Swayne, quoted by Withering, 1796; Knapp, 1804) owing to their robust habit, and it is generally agreed that such plants are particularly vigorous. Since they are perennial, this vigour presumably leads to exceptional persisting capacity, so that in any area the *F. loliacea* plants present may vary greatly in age. Schultz (1854) further urges that they increase rapidly by means of rhizomes. This statement appears to be an exaggeration, but it is evident that the regular production of relatively few hybrids over many seasons, or of a large number in particular seasons, would be sufficient to account for the prevalence of the plants.

It is curious to note that while many writers have discussed the question of the parentage of *F. loliacea*, few have speculated as to the direction in which the cross occurs in nature. Ascherson (1864) is an exception, but he was interested, at least primarily, from the point of view of proper nomenclature. The present results show that hybrids can be produced when either *F. pratensis* or *Lolium* acts as the pistillate parent.

The significance of the present results in relation to chromosome numbers will be discussed later, but it may be noted that they are of very considerable interest for two reasons. (1) Although each is a diploid species and reciprocal hybrids can be produced, the cross has proved to be one of the most difficult to accomplish successfully under artificial conditions. (2) The type of caryopsis produced is altogether different according to whether *L. perenne* or *F. pratensis* is used as the female parent.

Cross II. *Lolium temulentum*¹ ♀ × *Lolium perenne* ♂.

Attempts to intercross these two species were made in 1922, and the results have been described elsewhere (Jenkin, 1924). No further attempts have been made, but since the publication referred to is now out of print, the results may be briefly stated.

¹ The somatic chromosome number of this species is given by Faworski (1927) as 14, and this has been adopted for the purposes of the present paper.

L. temulentum is normally self-pollinated, but although no exerted stigmas were observed in the female unit first prepared, it was found that pollination of the emasculated spikelets gave stimulated ovaries. Two further units were then prepared in the same season, and an attempt was made to pollinate the florets of one of them by the method usually employed in the case of cereals, namely, by inserting between the paleae anthers which were on the point of dehiscing. There is no evidence that these anthers did in fact dehisce, nor that the stigmas were receptive, but in any case, no caryopses of any kind were produced, and the result therefore tallied with those obtained where a unit had been prepared but not pollinated.

The remaining unit was dealt with in exactly the same way as the first, namely, the emasculated spikelets were repeatedly dusted with pollen although again no exerted stigmas were seen. Swollen ovaries were again obtained.

The figures given in Table I refer only to two crosses which were made in the usual way, and from these a seed setting of 50 per cent. resulted. The caryopses were well developed but slightly shrivelled. Although it was ultimately found that one of them was the result of self-pollination, it was not sufficiently different from the others to be noticeably so.

On the incubator, no sign of germination was found except in this one seed, so that the cross gave a positive result only in so far as ovary stimulation and caryopsis development are concerned. There is also in this case definite evidence that the ovary stimulation was due to pollination with *L. perenne* pollen, since two emasculated units, one of which was pollinated by another method and the other left without pollination of any kind, failed to give even partially positive results.

Cross III. *Lolium perenne* ♀ × *Festuca ovina* ♂.

Two crosses of this type were made in 1930. The same *F. ovina* plant was used in both crosses. This plant belongs to a strain some of whose representatives have been investigated cytologically by Dr B. L. Sethi, a former research student at the Welsh Plant Breeding Station, who found that the gametic chromosome number is 14 and the somatic number 28. The cross is therefore of the type $2n = 14 \times 2n = 28$.

The pollination conditions were unsatisfactory, but a seed setting of 16.7 per cent. was recorded. Caryopsis development was generally poor but covered a rather wide range. In two of the "heavy seeds" the kernels closely approached the normal, and these, together with some others,

would be expected to germinate had they been produced by intraspecific pollination. Since they all failed to germinate, the only positive result concerning this pair of species is, that the pollination of *L. perenne* stigmas by *F. ovina* pollen may give rise to rather well-developed caryopses.

As far as I am aware, no natural hybrids of this parentage have been reported, and there is nothing in M'Alpine's account (1898) of Garton's work to show whether the attempts made by the latter were successful. It is extremely doubtful whether hybrids between plants of the types here employed can be expected to occur in nature, because the two species are found in close proximity only where there is a very abrupt change in habitat conditions, with *L. perenne* in a relatively fertile pasture on the one hand and *F. ovina* on a poor dry bank or rocky outcrop on the other. This perhaps does not apply to all types of *F. ovina*, but it certainly does with regard to this particular strain which originated from seed collected on a Ling heath.

Cross IV. *Glyceria fluitans* ♀ × *Lolium perenne* ♂.

Sinclair (1816) shows that at one time some botanists regarded *F. loliacea* as the natural hybrid between *G. fluitans* and *L. perenne*. The question was again raised in 1917 when the late Dr G. Claridge Druce collected some specimens which he and some other taxonomists considered to be hybrids between *G. plicata* and *L. perenne* (*B.E.C. Rep.*, 1918). Others disagreed, and found no reason why the specimens should not be classified as *F. loliacea*, implying that they were of the parentage *F. pratensis* with *L. perenne*. Through the courtesy of the late Dr Druce and the authorities of the Natural History Museum, South Kensington, I have been able to examine some of these specimens, and I agree that they are classifiable as *F. loliacea* Curt.

In 1931 I was able to pair *G. fluitans* and *L. perenne* in the manner shown above, using a female unit of 182 florets. These florets, after emasculation, exerted their stigmas perfectly and were thoroughly pollinated, but at harvest the ovaries did not show the slightest sign of stimulation.

In this case, although the cross has only once been attempted, the completely negative result, obtained under conditions as nearly ideal as the cool greenhouse will allow, becomes positive evidence, showing that pollination alone in this case does not lead to a development of the ovary, and, further, that when the cross is made in this particular direction, the two species appear to be very highly incompatible.

No information is available concerning the reciprocal cross.

Cross V. *Lolium perenne* ♀ × *Dactylis glomerata* ♂.

Cross VI. *Lolium perenne* ♀ × *Arrhenatherum avenaceum* ♂.

Each of these two crosses were attempted in 1921. The female units used were very small, but they were well pollinated. At harvest there was no indication in either case that the ovaries had been stimulated.

Cross VII. *Lolium perenne* with *Festuca arundinacea*.

The supposed natural hybrid referred to by Hackel (1882) as *F. elatior* × *L. perenne* is doubtless the *F. loliacea* of Curtis and therefore does not provide an instance of a natural hybrid of the type at present under discussion. Further, since it would appear that the references to a hybrid between these species given by Holmberg (N.D.), Saint-Yves (1929) and Andersen (1931) are all concerned with a single example recorded for Sweden, the taxonomic evidence that these two species intercross spontaneously in nature is extremely meagre.

According to M'Alpine (1898) two types of tall fescue were crossed by Garton with both *L. perenne* and *L. perenne multiflorum*, and inflorescences representing four types of cross are illustrated; but since, as already pointed out, no details are given, it is impossible to know exactly what results were obtained.

Cross VII a. *Lolium perenne* ♀ × *Festuca arundinacea* ♂. This type of cross was first made in 1921 and was repeated in 1928, 1929 and 1931. In 1928 the pollination conditions were poor, and a seed setting of only 12.7 per cent. was recorded. The highest seed setting, 60.5 per cent., was obtained in 1929, and 73.1 per cent. of the "heavy seeds" from this cross germinated. The unit was small, consisting only of 43 emasculated florets, so that a plant establishment of 18 represents practically 42 per cent. of the florets used, as compared with an average of 11 per cent. for all the crosses. This shows, on the one hand, that conditions, involving many different factors, have a very great effect upon the final results obtainable, while on the other it proves that under favourable conditions a relatively very high degree of success may be attained even in a cross involving two plants belonging to different genera, and also differing widely in chromosome numbers.

The best caryopses were in no case fully developed, although they were broad and plump in relation to their lengths, so that an average germination of 39.5 per cent. is surprisingly high. In vigour, the young seedlings were certainly well below those of intraspecific crosses and some were very weak, but even though some seedlings survived only as the result of careful nursing, the fact that 81 germinating seeds have given

75 established plants shows that F_1 hybrids between *L. perenne* and *F. arundinacea* can be obtained without very serious difficulty when the former is used as the pistillate parent.

The F_1 hybrids, when well established, are quite vigorous and are easily perpetuated vegetatively, but they are functionally male sterile.

Cross VII *b*. *Festuca arundinacea* ♀ × *Lolium perenne* ♂. The highest seed setting from this type of cross, 47.0 per cent., was recorded in 1929, and the lowest, 8.3 per cent., in 1930 when the female unit was inadequately pollinated. The average, as shown in the table, was 26.8 per cent. as compared with 32.9 per cent. when the cross was made in the opposite direction (Cross VII *a*). This difference cannot be held to be significant.

Caryopsis development was definitely better in the present crosses, and the best caryopses would be indistinguishable from those produced by intraspecific crossing. In marked contrast with those from the direct cross, however, all the seeds failed to show any signs of life on the incubator, so that no seedlings have so far been obtained.

Since the present crosses have been repeated in both directions in several seasons, and the results, although somewhat variable in seed setting, are consistent in other respects, it is clearly proved that under the conditions of the experiments the type of result obtainable depends upon the direction in which the cross is made.

Although established F_1 hybrids of the type *L. perenne* ♀ × *F. arundinacea* ♂ can be obtained without very serious difficulty, the seedlings are definitely less vigorous than those obtainable from intraspecific crossing, and at best, those actually produced in the present experiments would have little chance of surviving in severe competition. Supposing, however, that in nature, perhaps only in particular localities or in rare seasons, the conditions are more favourable for caryopsis development, seed germination and plant establishment, there seems to be no reason why F_1 seedlings of this type should not become established. Plants of the two species rather frequently occur in somewhat close association, and possibly therefore such hybrids may be more numerous than the taxonomic records would suggest, since again there is the possibility that they may have been overlooked owing to some similarity to certain types of *F. loliacea* and of *F. pratensis*.

Cross VIII. *Festuca pratensis* with *Festuca arundinacea*.

A certain amount of confusion with regard to the proper classification of these two species has long existed, although typical examples present no serious difficulties either in the vegetative or in the flowering growth

stages. Hackel (1882) places both as subspecies of *F. elatior* L.—a classification which makes it difficult or impossible to know with certainty which type he has in mind when, for instance, he describes one parent of a supposed hybrid simply as *F. elatior*.

The confusion is well illustrated in M'Alpine's account (1898) of Garton's work. Three types of broad-leaved fescues are given separately as *F. pratensis* (meadow fescue), *F. elatior* (tall fescue) and *F. arundinacea* (New Zealand tall fescue), and similar classifications may still be found in seedsmen's catalogues. What they actually stand for it is impossible to decide, but from an examination of plants grown from seed obtained under the various descriptions it would appear that there is no essential distinction between the "tall fescue" and the "New Zealand tall fescue" of commerce, although the latter may be, on the average, a somewhat coarser type.

The *F. pratensis* and *F. arundinacea* plants mainly used in the present crosses are of local origin and may be truly indigenous. They are all typical, and present no difficulties of classification. Representative plants were investigated by Evans (1926), who found that in *F. pratensis* the gametic chromosome number is 7, while the somatic number in *F. arundinacea* is about 40, and the gametic number 21. These results have been confirmed for typical plants by other workers, but it should be mentioned that in a plant classified as a variety of *F. pratensis*, Stählin (1929) found the somatic number to be 42. He also reports that the variety *Uechtritiziana* of *F. arundinacea* differs from the type and also from *F. pratensis* in having the somatic number 28. Further, Levitsky and Kuzmina (1927) have reported $2n = 70$ for two varieties of *F. arundinacea*.

These aberrant results do not in any way affect the present discussion, but they may possibly have some bearing upon the taxonomic, and particularly the agricultural, confusion that has existed. It is extremely doubtful, however, whether these types are very widely distributed, and confusion is more likely to arise from the fact that within each of the two better known types there is a wide range of growth forms, so that many plants in a large collection from sources other than seeds grown especially for commercial purposes may give the impression that they are intermediates. As a rule, however, even such plants can, by close examination, be classified as one or the other of the two species without serious difficulty.

Hackel (1882) has provisionally classified a certain plant as subsp. *pratensis* var. *genuina* subvar. ? v. var. ? *intermedia*. Morphologically,

he considers this to be exactly intermediate between the two subspecies, and he queries it as a hybrid, although apparently he did not know whether the plant was sterile. This plant is considered by Ascherson and Graebner (1900-2) to be equivalent to their *F. pratensis* \times *F. arundinacea* and to the *F. arundinacea* \times *F. elatior* of Haussknecht. Holmberg (N.D.) shows that hybrids between the two species have been recorded from Sweden, Norway and Denmark, while Andersen (1931) gives further records for the last-named country. Radeloff (1930) also states that he has examined three specimens in the Berlin Botanical Museum.

Cross VIII a. *Festuca pratensis* ♀ \times *Festuca arundinacea* ♂. Four individual crosses of this type have been made, two in 1924 and two in 1928, and the aggregate results are shown in Table I. From 235 emasculated florets, 34 established plants (= 14.5 per cent.) were eventually obtained. The results for the four crosses vary considerably in detail. With an average seed setting of 53.6 per cent. for all four crosses, the range was from 32.4 to 94.6 per cent. Therefore, under favourable conditions, seed setting may be practically perfect. At the same time most of the caryopses obtained from three of the four crosses appeared to be perfectly normal, so that a germinating capacity of only 39.2 per cent. is far lower than would be expected from similar caryopses derived from intraspecific crossing. It is therefore evident that the degree of caryopsis development reached is not a criterion of germinating capacity even in crosses where a proportion of the seeds germinate quite well. Quite possibly in a case of the present kind germinating capacity depends upon the condition of the embryo rather than upon endosperm development¹.

Germination was, on the whole, rather strong, so that from 40 seeds that showed signs of germination 34 established plants were obtained. All the seedlings from one of the crosses survived. This cross actually gave 94.6 per cent. seed setting, 60.0 per cent. germination, and 100 per cent. seedling survival, so that practically 57 per cent. of the florets emasculated gave established *F*₁ plants. Thus, under favourable conditions, it is clear that these two species, with *F. pratensis* as the female parent, can give very high results from intercrossing.

One of the crosses made in 1928, on the other hand, gave very poor results. Seed setting only reached 32.4 per cent. and caryopsis development was generally poor. Even in this cross, however, some seeds were

¹ It should be noted that germinating capacity is throughout judged, not by the capacity of the seeds to produce a seedling that can appear through the soil, but by their capacity to show even the slightest evidence of life on a filter-paper pad in an incubator in the laboratory. Many of the germinations recorded would have been missed altogether under soil conditions.

quite well developed, but they all failed to germinate. For this cross, an *F. pratensis* plant not otherwise used in these experiments was employed. It was easily classifiable as *F. pratensis*, although differing somewhat from the "standard" plants used. When intercrossed with such "standard" plants, it gave normal intraspecific results. Failure in this case therefore seems to have been caused by either (1) individual plant incompatibility or (2) unfavourable conditions for seed setting and caryopsis development.

Cross VIII *b*. *Festuca arundinacea* ♀ × *Festuca pratensis* ♂. Three crosses of this type were made in 1924, and a single cross in 1928. In the 1928 cross, an "outside" *F. arundinacea* plant, definitely classifiable as such although differing somewhat from the "standard" plants, was used. It gave a higher seed setting percentage than the lowest of the 1924 crosses (41.5 as compared with 34.0), but caryopsis development was very poor and none of the seeds germinated. The result is therefore very similar to that of the VIII *a* cross discussed above.

The aggregate results for all four crosses do not differ materially at any point from those obtained when the cross was made in the opposite direction (VIII *a*). On the whole, however, caryopsis development was somewhat less perfect in the present case, but this did not adversely affect germinating capacity nor plant establishment. In fact, one of the present crosses gave a germination of 72 per cent. as compared with 60 per cent., so that again caryopsis development is shown not to be a good criterion of germinating capacity. On the other hand, the highest seed setting now only reached 67.6 per cent. as compared with 94.6 per cent., but since seed setting may easily be affected by the pollination conditions, this fact cannot be held to be significant.

The established F_1 plants show no marked differences either in vigour or in morphological characters according to the direction in which the cross is made, and they are all functionally male sterile.

Therefore, despite the fact that we are here dealing with a combination of the $2X + 6X$ type, there is no evidence that the direction in which the cross is made has any material influence upon the result except possibly in respect of endosperm development.

Plant establishment in relation to the number of germinated seeds shows an average loss of about 37 per cent. of the seedlings. It is perhaps necessary here to point out that this loss of seedlings in all the crosses here under consideration appears to be due to extreme weakness, and occurs at any point up to the third leaf stage. Observation on pure albino seedlings from intraspecific crosses has shown that such albinos behave in

the same way, so that the cause of death appears to be the exhaustion of the reserve food supply in the seed on the one hand, and failure of the seedling to find its own nourishment on the other. These losses are therefore probably different from those referred to by Christoff (1928) and by Kostoff (1930) in *Nicotiana* seedlings, especially since surviving and non-surviving seedlings occur in the same family.

The loss of about 37 per cent. of the seedlings therefore is an indication of the general vigour of the plants, since losses of normal green seedlings in intraspecific crosses are always very small. Even the strongest seedlings were relatively weak, so that very favourable conditions would be necessary before they could become established in the field.

The two species differ somewhat in their habitat requirements, but they are not infrequently found growing fairly closely together and sometimes in the same pasture. The opportunity for natural crossing therefore probably occurs relatively often, and it is possible that natural hybrids are produced much more freely than the records would suggest, particularly since it would be quite easy, in the absence of very careful observation to classify true F_1 hybrids as forms of *F. arundinacea*. In fact, it may be suggested that the *F. elatior* referred to by Curtis (1798) and the *F. elatior* var. *sterilis* of Sinclair (1816) may have been hybrids of the present type.

Cross IX. *Festuca pratensis* ♀ × *Festuca gigantea* ♂.

The *F. Schlickumi* of Grantzow is supposed to be the naturally produced hybrid between these two species. It is represented as *F. pratensis* × *gigantea* by Ascherson and Graebner (1900-2), while Holmberg (N.D.) reverses the order¹.

So far, I have only attempted the cross in the direction shown above. Seed setting at 60.9 per cent. is quite good, if, as is necessary in practically all cases, we allow that pollination was not perfect. Caryopsis development was, however, on the average decidedly poorer than in the *F. gigantea* with *F. arundinacea* crosses described below, although it appeared to be perfect in 12 seeds out of 28. The remaining 16 seeds graded somewhat, but in most the caryopses were again normal or nearly normal in length. They were, however, rather thin and flattish (rather than slender and shrivelled). Only eight of the better developed caryopses and 15 of the others were incubated. The former gave full germination, but the

¹ It is impossible to know whether in any of these cases any special significance is to be attached to the order in which the species are given, although if this were of no importance, there is no reason why the order should be changed.

seedlings were somewhat weak, and two of them failed to reach the established plant stage. Fifteen of the less well-developed seeds were also incubated, but none of them germinated. Actual germination in this case was therefore 34.8 per cent., but allowing for the seeds which were not sown, the total germinating capacity may be estimated at 42.9 per cent.

Except in the matter of seed setting therefore this cross was throughout less successful than those represented under XIV *a* and XIV *b* below, but it was yet by no means unsuccessful, and some of the seedlings produced might be expected to become established even under natural conditions provided competition were not particularly severe. However, the opportunity for the intercrossing of these two species probably does not often occur in nature, since the difference in habitat requirements is apparently quite considerable.

The *F. pratensis* plant used in the present cross belongs to a strain investigated by Evans (1926), who found reason to believe that the gametic chromosome number is 7. The plant is typical of the species, and for such plants the somatic number 14 has been recorded by Levitsky and Kuzmina (1927), Stählin (1929) and Radeloff (1930). Assuming therefore that *F. gigantea* is of the type $2n = 42$ (Stählin, 1929; Radeloff, 1930), the present cross can be represented by $2n = 14 \times 2n = 42$. While this cross has been less successful than the type $2n = 42 \times 2n = 42$ discussed below (Cross XIV), it has yet been a distinct success, and the established plants obtained are vigorous. They also are functionally male sterile.

Whether the cross can be made in the opposite direction with *F. gigantea* as the female parent is not known.

Cross X. *Lolium perenne* with *Festuca rubra*.

Holmberg (N.D.) refers to a plant formerly described by him as the spontaneously produced hybrid *F. rubra* \times *L. perenne*. Later, however, he withdrew this determination in favour of *F. pratensis* \times *L. perenne*, so that the only record of which I am aware of a natural hybrid between the present species has been cancelled.

Cross X *a*. *Lolium perenne* ♀ \times *Festuca rubra* ♂. The aggregate results for three crosses of this type are shown in Table I. The *F. rubra* plant used in each case was B1-299, but three different *L. perenne* plants were employed. The gametic chromosome number of the *F. rubra* plant has been found to be 21,¹ so that the cross is of the type $2n = 14 \times 2n = 42$.

¹ For this information I am indebted to Dr F. H. Peto, a former research student at the Welsh Plant Breeding Station.

The variation in seed setting from one cross to another was considerable, but this must be due either to the conditions or to variation in compatibility of the *L. perenne* plants with the *F. rubra* plant B1-299. In each case, however, germinable seeds were obtained, while each cross also gave rise to established F_1 hybrids. Seed setting at an average of 45.4 per cent. was quite definitely high, while a maximum of 74 per cent. was reached in one of the crosses. Caryopsis development was also remarkably good, and although there was a considerable range, the best caryopses would be indistinguishable from those of pure *L. perenne*. Germination at 18.2 per cent. was therefore very low and the seedlings were generally very weak, so that even with careful nursing only nine established plants were ultimately obtained from 14 germinating seeds. Some of these F_1 hybrids are quite strong, but others have remained relatively weak. They closely resemble the male parent morphologically, but are all functionally male sterile.

Cross X b. *Festuca rubra* ♀ × *Lolium perenne* ♂. The reciprocal cross has also been attempted three times, the one made in 1928 being the full reciprocal of the direct cross of the same year. In this season and in 1921 the *F. rubra* parent was again plant B1-299, but in 1922 an unrelated *F. rubra* plant of unknown chromosome number was used. In contrast with the direct crosses, *L. perenne* ♀ × *F. rubra* ♂, seed setting was very low, and caryopsis development very poor, rarely reaching half the length of the paleae. These caryopses were also very slender and shrivelled so that complete failure to germinate was not unexpected.

The results for the direct and the reciprocal crosses were therefore quite different according to whether *L. perenne* or *F. rubra* was used as the female parent, and in this respect the present combination agrees with that for the similar chromosomal combination *L. perenne* with *F. arundinacea* described above. In both cases, established plants have been obtained when *L. perenne* ($2n = 14$) was used as the female parent, but the seedlings were stronger when *F. arundinacea* served as the pollen parent. In both cases also, the crosses failed when *L. perenne* was used as the male parent, but whereas the caryopses were well developed when *F. arundinacea* was the pistillate parent, they were very poor when *F. rubra* was employed in a similar capacity. Thus, while in some respects the results for the two combinations agree, in others they differ quite distinctly.

The seedlings produced in the present case would probably have no chance of surviving under natural conditions, but they were certainly no weaker than those obtained from *L. perenne* with *F. pratensis* (Cross I

above), and since it is generally supposed that spontaneous crossing in the latter pair gives established plants, it might be expected also that hybrids of the type *L. perenne* with *F. rubra* might also occur in nature. Such hybrids have not been recorded, but it is still quite conceivable that they do exist, since the two species frequently occur in the same habitats, and the F_1 hybrids might quite easily be overlooked or else classified as types of *F. rubra*. In fact, it is difficult to find a single morphological character in which the artificially produced hybrids show quite unmistakably the influence of the *L. perenne* parent.

Cross XI. *Festuca pratensis* with *Festuca rubra*.

The only reference to the occurrence of a natural hybrid between these two species of which I am aware is that made by Saint-Yves (1929). The authority for the determination is given as Wein in *Fedde Repert. nov.sp.* (1909). I have been unable to consult this reference, but Saint-Yves is obviously very doubtful whether the classification can be accepted.

Cross XI a. *Festuca pratensis* ♀ × *Festuca rubra* ♂. This particular cross has been attempted five times, two of the attempts having been made in 1931 and the others in 1922, 1928 and 1930 respectively. In the 1922 and 1928 crosses, the *F. rubra* plant B1-299 was again used, while the corresponding parent in 1930 was derived from B1-299 by self-pollination. These three crosses, therefore, are presumably of the $2n = 14 \times 2n = 42$ type. In 1931, two different *F. rubra* plants, B1-1029 and B1-1044, were employed. The former is again of the $2n = 42$ type, but the latter has a somatic chromosome number of 56.¹

Had the results obtained by the use of plant B1-1044 been in any way appreciably different from the others, it would be necessary to tabulate them separately, but the variation found is so slight that it could easily be accounted for by causes other than difference in chromosome number.

Seed setting at 10.6 per cent. was definitely low. The caryopses obtained within each cross varied considerably in development, reaching from just below half the length of the paleae to practically normal length. Most of them were somewhat shrivelled, but some appeared to be normal. None of the seeds showed the slightest signs of life on the incubator.

Cross XI b. *Festuca rubra* ♀ × *Festuca pratensis* ♂. Five crosses of this type have also been made. The *F. rubra* plant B1-299 was not used, but

¹ I am indebted to Dr B. L. Sethi (1931) for information concerning B1-1029 and B1-1044.

two derivatives of it by self-pollination were employed respectively in 1929 and 1930. These two may probably be assumed to be of the type $2n = 42$. Plants B1-1029 ($2n = 42$) and B1-1044 ($2n = 56$) were again used in 1931 as well as another *F. rubra* plant, B1-1036, which has not been investigated cytologically.

The two crosses in which B1-1029 and B1-1044 were used both failed to set any seed from female units consisting respectively of 92 and 86 emasculated florets. Failure on the part of B1-1044 was therefore not necessarily due to its chromosomal constitution.

The remaining three crosses gave very uniform results, with an average seed setting of 20.9 per cent., but the average seed setting for all five crosses was not appreciably higher than in the direct cross (XI a above). Caryopsis development was quite definitely poorer when *F. rubra* was used as the female parent, rarely reaching beyond half the length of the paleae. These caryopses were, however, flattish and thin, rather than slender and shrivelled. All the seeds failed to germinate.

From the combination *F. pratensis* with *F. rubra*, therefore, no germinable seeds have yet been obtained, although definite caryopsis development followed pollination whether one or the other species was used as the female parent. The data available are insufficient to show whether the two chromosomal types of *F. rubra* are capable of giving different results when used with *F. pratensis*, but it is shown that both the $2n = 42$ and the $2n = 56$ types can give stimulated ovaries when used as male parents with *F. pratensis*. It has not been proved, however, that the $2n = 56$ type can give stimulated ovaries when used as the female parent.

There is no doubt that in this combination the results obtained differed quite definitely according to the direction of the cross, particularly in the size of the caryopses. The type of caryopsis also appears to be different, although this is certainly less pronounced than in the combination *L. perenne* with *F. pratensis*. The best developed caryopses were obtained when the species with the lower chromosome number was used as the female parent.

As far as chromosome numbers are concerned, this cross is comparable with Cross X above, where *L. perenne* and *F. rubra* were the species employed. With *F. rubra* as a common male parent, seed setting was much higher in *L. perenne* than in *F. pratensis*, but the difference in caryopsis development was less marked although it was still in favour of *L. perenne*. This, possibly, is the reason why germination, leading to established plants, was obtained in the one case and not in the other,

although it has been seen that in other crosses the degree of caryopsis development is not necessarily a criterion of germinating capacity.

When *F. rubra* was used as a common pistillate parent, *L. perenne* and *F. pratensis* gave very similar results, so that on the whole the two species behaved very similarly when paired with *F. rubra*, with the exception that germinable seeds and established plants have only yet been obtained when *L. perenne* was used as the female parent.

These results for *F. pratensis* with *F. rubra* are positive only in so far as caryopsis development is concerned, so that it still remains to be shown that interspecific hybrids of this type are possible. Yet, the impression the work has left is that should it be possible to improve the conditions only to a relatively small extent, germinable seeds might be produced, since, although some apparently very well-developed caryopses have been obtained, they were perhaps not sufficiently numerous to give a good representation of the possibilities of the cross; and it is known that in some other crosses only a proportion even of the apparently well-developed seeds are capable of germination.

Cross XII. *Festuca ovina* with *Festuca rubra*.

Under the general heading "*Hybrides douteux ou plantes nullement hybrides*" Saint-Yves (1929) refers to " $\times F. Zobelii$ Wein in *Fedde Repert. nov. spec.* (1909) = *F. ovina* \times *F. rubra* (loc. cit.)." He believes not only that this combination is possible but that he has examined a hybrid plant of this type.

Cross XII a. *Festuca ovina* ♀ \times *Festuca rubra* ♂. Three crosses of this type were made in 1930. The *F. ovina* plants used again belonged to the strain already referred to and in which Dr Sethi found the gametic and somatic chromosome numbers to be respectively 14 and 28. Unfortunately none of the *F. rubra* plants employed as male parents has been examined cytologically, but they are all obviously classifiable, using Hackel's classification (1882) as *F. rubra* subsp. *eu-rubra* var. *genuina*.

It has already been mentioned that in *F. rubra* Dr Sethi found two types, in one of which the gametic and somatic chromosome numbers were respectively 21 and 42, while in the other the corresponding numbers were 28 and 56. These two types have also been reported by other workers even within the subspecies *eu-rubra* of Hackel (1882). It seems probable, however, that the great difficulty that exists in the ultimate classification of *F. rubra* types accounts for the fact that whereas Levitsky and Kuzmina (1927) give the somatic number of *F. rubra* subsp. *eu-rubra* var. *genuina* Hack. as 56, Stählin (1929) reports the corresponding number to

be 42 in *F. rubra* subsp. *eu-rubra* var. *genuina* Hack. subvar. *vulgaris* Hack. At least, the fact that different chromosome numbers have been reported for two plants which differ taxonomically, according to these authors, only in subvarietal characters is sufficient to arouse suspicion either with regard to the determinations or with regard to the value of the ultimate systematic divisions.

No other numbers appear to have been reported for the subspecies *eu-rubra*, and four types investigated by Stählin in this subspecies agreed in being of the type $2n = 42$.¹

Although the chromosome numbers have not been determined for the *F. rubra* plants used in the present combination, it therefore seems highly probable that they are either of the hexaploid or the octoploid type.

No importance can be attached to the low rate of seed setting recorded, since the pollination conditions were very unfavourable. All the caryopses were also poorly developed, but three out of seven germinated and two established plants have been produced. Both these are of normal vigour and unmistakably show the influence of the male parent, *F. rubra*.

Cross XII b. *Festuca rubra* ♀ × *Festuca ovina* ♂. Only two crosses of this type, involving 132 emasculated florets, have been tried. The pollination conditions were again unsatisfactory, but since they were certainly better than those of Cross XII a better results were to be expected. Yet, as far as the meagre data are able to show, seed setting was now somewhat lower, while none of the three heavy seeds obtained was able to germinate, although caryopsis development in one of them was fairly good.

The chromosome numbers of the *F. rubra* plants used are again unknown, but for one of the two crosses the same pair of plants that had ultimately given two germinable seeds when mated in the reverse direction was again used.

The results obtained from this combination *F. ovina* with *F. rubra* are so meagre that they cannot bear the weight of serious argument. They are sufficient to show, however, that provided the opportunity occurs and favourable conditions obtain, the F_1 hybrids can be produced when *F. ovina* serves as the pistillate parent. Typically, the two species occupy rather definitely different habitats, but actually the opportunity for intercrossing should occur fairly frequently in nature since the required

¹ For the subspecies *violacea* (Gaud.) Hack., Stählin reports a somatic number of 14, and for subsp. *nevadensis* Hack. var. *Hackelii* Lit. et Maire, subvar. *brevifolia* Lit. et Maire, Levitsky and Kuzmina (1927) report the somatic number 70, but it is extremely improbable that these results in any way affect the present discussion.

habitats are frequently contiguous, and in the intermediate zone *F. ovina* may be found in the open ground and *F. rubra* in more shaded positions.

Cross XIII. *Festuca arundinacea* with *Festuca rubra*.

As far as I have been able to ascertain, natural hybrids between these two species have not yet been recorded.

Cross XIII a. *Festuca arundinacea* ♀ × *Festuca rubra* ♂. Three crosses of this type have been attempted. In two of these, the *F. rubra* plant, B1-299 (2*n* = 42), was again used, and in the third cross one of its derivatives by self-pollination. The results for the three crosses varied somewhat, but the variation is probably to be accounted for by differences in the conditions. Seed setting was low in each case, with a maximum of 16.7 per cent. in 1928, but caryopsis development was quite good, varying usually from more than half to two-thirds the length of the paleae. Most caryopses were also plump in relation to their length, but some were rather shrivelled. Yet, as in the case of similarly well-developed seed in some other crosses, no germination was obtained.

Cross XIII b. *Festuca rubra* ♀ × *Festuca arundinacea* ♂. Four crosses of this type have been made, three of them in 1921 and the remaining cross in 1928. In the former, the *F. rubra* plant B1-299 was again used, and, in 1928, one of its derivatives from self-pollination.

Seed setting was rather consistently higher than when the cross was made in the opposite direction, ranging from 11.8 per cent. in the 1928 cross to 60.0 per cent. in one of the others. Caryopsis development was, however, definitely poorer on the average, the kernels being, even at their best, much less plump in relation to their length. Yet seven seeds out of 91 (= 7.7 per cent.) germinated as compared with no germination from 22 seeds from the opposite cross.

The seedlings were very weak, but all seven were kept alive to become established on their own roots. They made very poor progress during the first summer and only one plant survived the first winter. This plant is still available but can only with great difficulty be perpetuated vegetatively from year to year. It has not yet produced an inflorescence.

There appears to be a definite difference in the result obtainable from this combination according to the direction of the cross, particularly in seed setting, in caryopsis development, and in germinating capacity, and it may be noted that whereas the better developed caryopses with *F. arundinacea* as the female parent failed to germinate, some germinating capacity was found in the reciprocally produced seed although the caryopses were definitely poorer.

The seedlings obtained would be too weak to survive under natural conditions of competition, so that naturally produced hybrids of this type can only be expected where or when the conditions are much more favourable than those of the present experiments. It has been shown, however, that the cross itself is possible.

Cross XIV. *Festuca gigantea* with *Festuca arundinacea*.

A single cross has been made in either direction, the same pair of plants being employed. The two plants were typical of their species, and in the absence of observations on the plant itself, the *F. gigantea* representative is assumed to be of the chromosomal type, $2n = 42$ (Stählin, 1929; Radeloff, 1930). The *F. arundinacea* plant belongs to the line investigated by Evans (1926) when he found the gametic chromosome number to be 21 and the somatic number about 40. The somatic number for typical *F. arundinacea* plants has since been reported by Levitsky and Kuzmina (1927) and by Stählin (1929) to be 42.

Since it may be assumed, following Ascherson and Graebner (1900-2), that the *F. Schlickumi* of Grantzow is the supposed hybrid between *F. pratensis* and *F. gigantea*, the only references to naturally occurring hybrids between the present species known to me are those given by Saint-Yves (1929) and Holmberg (N.D.). The latter states that hybrids have been found in three localities in Sweden and in a single locality in Denmark.

Cross XIV a. *Festuca gigantea* ♀ × *Festuca arundinacea* ♂. As shown by Table I, 74 per cent. of the emasculated florets used in this cross yielded heavy seeds. In 70 out of a total of 77 "heavy seeds" the caryopses appeared to be perfectly developed. Fifty of these were sown and they all germinated, giving rise to seedlings not appreciably weaker than those obtainable from intraspecific crosses, so that a full complement of established plants was obtained. In the remaining seven seeds, caryopsis development was somewhat below normal and only one of these germinated. Actual germination therefore is recorded as 89.5 per cent., but it is highly probable that the 20 seeds which were withheld would also have germinated, so that the estimated total germination reaches 92.2 per cent. This compares quite favourably with many results obtainable from intraspecific breeding¹.

Cross XIV b. *Festuca arundinacea* ♀ × *Festuca gigantea* ♂. The low seed setting percentage shown for this cross in Table I is not significant.

¹ The average germinating capacity of 9091 *L. perenne* seed obtained in 1929 and 1930 by intraspecific crossing was 96.8 per cent. (Jenkin, 1931 b).

The pollen mainly used was stale and therefore unsatisfactory. The seven caryopses obtained were again apparently perfect and ultimately gave rise to seven established plants. The seedlings were vigorous, and the established plants, as in the direct cross, were functionally male sterile.

The F_1 hybrids produced by intercrossing *F. arundinacea* and *F. gigantea* do not differ materially in morphological characters whether one or the other species be used as the female parent. Although the present results do not prove that this is the case for seed setting, it is probable that there is again no difference either in this respect or with regard to caryopsis development or seed germination.

While the parent species agree in chromosome numbers, they are morphologically so dissimilar that they cannot even be regarded as extreme forms of the same species. In fact, *F. gigantea* Vill. has often been known as *Bromus giganteus* L.

The ease with which the two species can be intercrossed under artificially controlled conditions suggests that natural hybrids should be far more abundant than the meagre records available would seem to indicate. It is possible that such hybrids have been overlooked, but the normal distribution of the two species is so different that the opportunity for intercrossing probably occurs very rarely. *F. gigantea* is typically a shade-loving plant and is usually found under the shade of trees, while *F. arundinacea* is definitely less shade-loving, although it occurs mainly on heavy soils and under moist conditions. Here, therefore, the production of natural hybrids is probably limited by conditions other than lack of compatibility between the two species.

GENERAL DISCUSSION.

(1) *The results in relation to spontaneous crossing.*

This aspect of the present results has already been discussed under each species combination described. It has been emphasised in dealing with the combination *L. perenne* with *F. pratensis* (Cross I) that the conditions of the present experiments may differ materially from those obtaining in nature, and that for this reason the present results may not represent exactly what happens in the field. This applies also to all the other crosses described, but it is to be expected that the difference between the natural and the experimental conditions would not affect all crosses in precisely the same way nor exactly to the same extent. Where, however, fully positive results have been obtained under greenhouse conditions, it can be concluded that provided, in the aggregate, the conditions in nature are at any time equally favourable successful spontaneous crossing

between each pair of species concerned may also occur in nature. Further, it would appear that if in certain cases the conditions are only slightly more favourable in nature, other types of F_1 hybrids which have not yet been produced artificially may also occur. Purely negative results under artificial conditions on the other hand are necessarily true only for the conditions described, and complete failure under such conditions cannot prove that in the field any particular cross is impossible. When negative results are persistent and consistent, however, or where they have been obtained under what appear to be optimum conditions, they do acquire a measure of positive value, since they probably indicate a very low degree of compatibility between the species concerned.

(2) *The results in relation to taxonomy.*

In his report of the work carried out and the results achieved by Mr John Garton, M'Alpine (1898) wrote: "These creations of Mr Garton will likely lead botanists to fuse the two genera *Lolium* and *Festuca* into one." This forecast has not yet been realised, nor is it expected or intended that the present results should have any such effect, whatever their bearing upon the true relationships of these genera may be. Whether this change be desirable or not must depend upon our conception of the functions of taxonomy. Fundamentally, this science is concerned only with classification based upon morphological and possibly physiological characters which are constant and capable of exact definition and description. Consequently taxonomic position does not necessarily carry any phylogenetic implications, and however closely allied phylogenetically the two genera *Lolium* and *Festuca* may be, taxonomically the former properly belongs to the Hordeae and the latter to the Festuceae. It must be clearly understood, however, that the fact that the two genera are placed distantly apart taxonomically does not necessarily involve any phylogenetic implications. In so far as the genera *Lolium* and *Festuca* are concerned, therefore, the present results cannot affect the taxonomic position.

When, however, we come to more detailed classification the position is different. The great paucity of well-defined and easily described morphological characters in the Gramineae has undoubtedly proved a very serious difficulty in the work of ultimate classification. The confusion that has existed in the case of *F. arundinacea* and *F. pratensis* is doubtless mainly due to this cause. In this case cytological evidence has shown that those taxonomists who have regarded the two types as distinct species were probably right, and supporting evidence is found in the present

results. The ultimate classification of the fine-leaved fescues is notoriously difficult, and the very elaborate keys which exist have, at least in my own hands, proved more confusing than helpful. It may be that in these fescues inconstant characters have sometimes been given taxonomic values which they do not deserve, and that some of those regarded as of critical value for diagnostic purposes are subject to great differences of expression under different environmental conditions. Taxonomists perhaps would be most helpful in dealing with these fine-leaved fescues if they remained content with major classifications. If they wished to go further, it would seem very necessary that the material should be grown under conditions which are relatively uniform in order that the constancy of the characters themselves may be tested. Ultimate classification will then probably be left to the plant-breeder and the cytologist, but even then the results must as far as possible be correlated with morphological characters before they can be of any great use, particularly to the plant-breeder who is concerned with economic problems.

(3) *The results in relation to chromosome numbers.*

In some cases the chromosome numbers of either the particular plants used or of the lines or strains to which they belong have been investigated and determined by former research students at the Welsh Plant Breeding Station¹. In other cases the reported numbers as they appear in the literature have been accepted for the purpose of the present paper. It should be pointed out, however, that whereas Evans (1926) working on the *F. arundinacea* line based on plant bN-1 found the gametic chromosome number to be 21 and the somatic number about 40, while the gametic and somatic numbers for this species are given in the literature as 21 and 42 respectively, Peto (1933) found that in at least one plant used in the present experimental work the somatic number was 43—apparently 42 normal chromosomes and a fragment. Since all the *F. arundinacea* plants used in the present series of crosses have not been individually examined, it cannot be determined to what extent this fact may affect the results obtained. Where crosses have been repeated, however, various *F. arundinacea* plants have been used, so that the slightly abnormal chromosome conditions in one or a few plants taken at random have probably little effect upon the aggregate results obtained. This fact, however, must be borne in mind, and the possibility that it may have to some extent affected the results must also be allowed for. Meanwhile the results will be discussed as if such an abnormality were not involved.

¹ Mr Gwilym Evans, Dr B. L. Sethi and Dr F. H. Peto.

It will be noticed that in all of the species investigated the basic chromosome number is 7, but it is reasonable to believe that the two species *Dactylis glomerata* and *Arrhenatherum avenaceum*¹, both tetraploids, have yet little in common either with each other or with the other species of the series. Only two other tetraploids have been included, and these have been used only to a limited extent, so that the species mainly employed have been either diploids or hexaploids, with a few octoploids in the case of *F. rubra*.

(a) *Diploid with diploid*. Kihara and Nishiyama (1932) found that certain diploid *Avena* species can be easily intercrossed, and that the seed produced give full germination. Seed setting was, however, low in the two cases where *A. Wiestii* was used as the female parent, but this may be due to technical difficulties arising from the relatively small size of the florets. The kernels produced in all cases are stated to present "about the same appearance as those of the mother plants."

The wild and cultivated species of *Secale* are also stated to intercross quite easily, and in one case F_2 progeny plants have been studied (Tschermak, 1914; Ossent, 1930).

Direct references to diploid-diploid crosses are not given by Sax (1921, 1922), but he states that the species within each of the three *Triticum* groups are interfertile, while Bleier (1930) makes a somewhat similar statement. That this is true for *T. monococcum* with *T. aegilopoides* is shown by Kihara, Wakakua and Nishiyama (1929), but their results suggest that the germinating capacity of the seed differs according to the direction of the cross. Wakakua's results, quoted by Kihara and Nishiyama (1932), show that both seed setting and seed germination were high in "einkorn" with "einkorn" crosses, but the species employed are not specified.

Thompson (1926) states that "einkorn" cannot be crossed with rye, although according to Percival (1921) Tschermak was successful in obtaining a hybrid from a cross between *Triticum monococcum* and *Secale cereale*. I have, however, been unable to find any references to hybrids between diploid representatives of *Aegilops*, or to intergeneric diploid crosses between *Triticum* and *Aegilops*.

Many references to hybrids between *Zea mays* and *Euchlaena mexicana* may be found in the literature, and both Emerson and Beadle (1930) and

¹ The numbers accepted for this species are those given by Aase and Powers (1926). Davies (1927) did not definitely determine the somatic chromosome number of the bulbous type, but he considered the number to be about 40. The discrepancy has no material bearing upon the present discussion, but it indicates that further work on various types of this species is desirable.

Mangelsdorf and Reeves (1931) state that the two species can easily be intercrossed. In fact, it has sometimes been supposed that the two species interbreed almost indiscriminately when growing in close proximity, but Collins and Kempton (1921) found little evidence in support of this view in the Mexican area where *Euchlaena mexicana* occurs as a weed in or near to maize fields.

Outside the Gramineae, Rasmuson (1921) and Chittenden (1928) have found that *Godetia amoena* and *G. Whitneyi* can be intercrossed in either direction. Similar results have been reported by Christoff (1928) for certain *Nicotiana* species. If, however, we regard as diploids the *Nicotiana* series in which the gametic chromosome number is 12, we find that the results obtained by Christoff vary considerably even when species of this group are intercrossed amongst themselves. The results may vary not only from one species combination to another, but in five combinations out of ten they also differ more or less according to the direction in which the cross was made. The outstanding combination of the series is that of *N. glutinosa* with *N. sylvestris*. When the former served as the female parent, mature hybrids were produced; but when the cross was made in the opposite direction, no capsules were formed. The result in this extreme case was therefore similar to that reported by Karpechenko (1924) for *Raphanus sativus* with *Brassica oleracea*, while Müntzing (1930) failed to obtain positive results from one diploid-diploid cross in *Galeopsis* when the cross was made in either direction. Other results reported by Müntzing for this genus show very considerable differences from one species pair to another. While some pairs could be easily intercrossed, others yielded F_1 hybrids only with considerable difficulty. Laibach (1929) also found that *Linum perenne* with *L. austriacum* gave seed in which the embryos reached different stages of development according to the direction in which the cross was made, but by using special methods, involving the removal of the young embryo at an early growth stage followed by artificial nursing, he was able to obtain established plants from the reciprocal crosses.

Two diploid-with-diploid combinations are included in the present series. One of these, *Lolium temulentum* with *L. perenne*, has only been tested in one direction. No germinable seeds were obtained with the former as the female parent, although the caryopses were rather well developed. It is therefore evident that this combination differs in some essential respect from the wheat and oat combinations referred to above.

The other diploid-diploid combination, *L. perenne* with *F. pratensis*, is of greater interest, since the cross has repeatedly been attempted in

both directions. The results not only show that hybrid plants are difficult to obtain, but that the type of caryopsis produced differs fundamentally according to the direction in which the cross is made. With *F. pratensis* as the female parent, the caryopsis is typically long, slender, shrivelled and often deformed. After about three weeks on the incubator such caryopses (in seeds which have failed to germinate) are found to be more or less swollen but not fully distended, and to contain practically nothing but a watery fluid. On the other hand, the caryopses produced by *L. perenne* as the mother plant are typically short, varying from about half the length of the paleae down to a point where it is difficult to be quite sure whether the ovary has been stimulated. In those seeds which fail to germinate the caryopses are plump and firm but not hard. They contain no surplus water, while the endosperm appears to be well organised and contains an abundance of starch grains. In the better seeds the actual amount of such endosperm present is by no means negligible, and failure to germinate is presumably entirely due to the abortion or death of the embryo.

These two types of caryopsis are remarkably similar to those described by Kihara and Nishiyama (1932) for the diploid-hexaploid combination *Avena strigosa* with *A. fatua*, the caryopses obtained in the present case by the use of *F. pratensis* as the female parent corresponding with those obtained when *A. strigosa* was similarly used. Whether the course of events which led up to this final position in the two cases is also similar cannot be determined, but the endosperm in the *L. perenne* ♀ × *F. pratensis* ♂ seeds is so well formed that it suggests primary rather than secondary origin. Moreover, while Kihara and Nishiyama failed to obtain germinable seed from the long-shrivelled caryopses, germination was relatively good in the short-plump type. In the present case established plants have been obtained from both types, but germination in both cases was extremely low.

Descriptively at least, the results obtained by Watkins (1927) from the tetraploid-hexaploid combination, *Triticum turgidum* with *T. vulgare*, are also very similar to those at present under discussion, but in this case, in contrast with those of the *Avena* cross already referred to, the long-shrivelled grain type gave fair germination, although decidedly lower than the short-plump type.

Though these two types of grain are usually associated in a particular way with a difference in parental chromosome numbers, they can also be produced when the numbers are similar. It is true that in the present case, as far as the small numbers available are able to show, the "success of

the cross" does not vary according to the direction of the cross, or, if it does, it is in favour of the long-shrivelled type of caryopsis. Even so, the result clearly calls for some explanation other than that of a difference in chromosome numbers.

Actually, the results show that in exceptional cases germinable seeds¹ are produced when the present cross is made in either direction. This means that during the development of the caryopsis in these exceptional cases the embryo can develop up to a point where it is capable of entering into the resting stage, from which it can resume growth even after some months when the conditions are favourable. The condition of the endosperm in such seeds has not been ascertained, and it is possible that survival of the seed is not of necessity associated with relatively good endosperm development. In practically all cases germination is extremely weak with the first leaf very narrow and slender, and the difficulty experienced in establishing such seedlings shows that either the endosperm is very deficient or else that the young seedling is unable to make full use of the endosperm available. There is no reason to suspect that this weak germination is caused by difficulties within the embryo itself, since some of the established plants are of quite normal vigour. Presumably, therefore, the death of the embryo during the development of the caryopsis is due to some influence external to itself², unless, as suggested for the caryopsis as a whole in the case of *Phalaris arundinacea* ♀ × *Ph. tuberosa* ♂ (Jenkin and Sethi, 1932), the gametes which united to form the embryo were themselves lacking in vitality. One type of possible outside influence affecting the development of the embryo has also been suggested in the same connection, namely, physiological changes in the embryo-sac and its immediate environment. The direct influence of the mother plant as suggested by Laibach (1929) and by Kostoff (1930), or lack of harmony in the physiology of the embryo and the mother plant (Müntzing, 1930), may also interfere with the normal development of the embryo, causing retarded development or actual death.

In the present case, however, the essential difference between the reciprocal crosses is shown in the development of the endosperm. If we assume that in this respect the seeds which failed to germinate afford a true picture of the general position (and they certainly do so for the great majority of the seeds produced), then we find that when *F. pratensis* serves as the female parent, the mature seed contains practically no

¹ Seeds harvested in one season are usually incubated not earlier than the January of the following year.

² It is, of course, conceivable that an endosperm may develop in the complete absence of an embryo.

endosperm, or at least none that is sufficiently well organised to be recognisable as such. When *L. perenne* is used as the female parent on the other hand, the endosperm, however small it may be, appears to be well organised. The conditions which may affect caryopsis development as a whole, as suggested for *Phalaris arundinacea* ♀ × *Ph. tuberosa* ♂ (Jenkin and Sethi, 1932), may again be operative, but it is clear that when the mother plant is *F. pratensis* those caryopsis tissues which are of maternal origin usually respond fully. It is possible that the response is independent of endosperm development, or even of any great measure of nuclear division in the endosperm rudiments, although the general impression given by the mature caryopses in this case is that development is very similar to that described by Kihara and Nishiyama in the cross *Avena strigosa* ♀ × *A. fatua* ♂. They ascribe the very rapid initial increase to the overstrong stimulative effect of the male nucleus, but since this suggestion is speculative, defining neither the nature of the stimulus nor the manner in which it can act, we can accept it only as part of a scheme which brings most of the results available into a general relationship. In fact, it would be simpler and just as informative to state that the two sets of *F. pratensis* chromosomes fail to form an effective means of endosperm development when associated with a single set of *L. perenne* chromosomes. The actual cause of this failure is, of course, still obscure. It is conceivable that the development of the ovary and nucellus (the mother plant part of the caryopsis) responds too readily to the fertilisation stimulus, and that the rapid initial increase of the endosperm is a futile and misdirected effort to keep up with this extraordinary development, since it is not clear that at any time the fully extended fruit case is filled with endosperm which later degenerates. In that case, the result would be due ultimately to the abnormal response of the mother tissue to an unusual type of fertilisation, and the essential relationship would be between the mother plant and the quality of the chromatin.

When *L. perenne* is the female parent, a well-organised but usually very small endosperm is produced. Under Kihara and Nishiyama's scheme this poor endosperm would be ascribed to a weak activating stimulus. Development actually ceases at various growth stages in very much the same way as described for the cross *Phalaris arundinacea* ♀ × *Ph. tuberosa* ♂ (*loc. cit.*), and the same considerations possibly apply. The simplest explanation of the difference between the caryopses obtained from the reciprocal crosses appears to be that whereas the double set of *F. pratensis* chromosomes with a single set of *L. perenne* chromosomes reacting with the *F. pratensis* mother tissue fail to form an efficient means

of endosperm development, the double set of *L. perenne* chromosomes with a single set from *F. pratensis* in the tissues of *L. perenne*, though not fully effective, are at any rate more efficient. In fact, the smooth and hard caryopses formed in the latter case suggest that the response of the mother tissue in the present case is inadequate, and that the development of the endosperm is therefore actually impeded. A similar suggestion has been made by Müntzing (quoted by Kihara and Nishiyama, 1932). This, however, does not seem to be the case in the early stages of development, since in some of the smaller caryopses the minute endosperm is found to occupy less than the entire volume of the fruit case, and cessation of development is therefore not due to mechanical restriction. That the direct action of the mother plant has a definite effect by cutting off or diminishing the nutritional supplies to developing seeds is suggested by the observations of Mangelsdorf and Reeves (1931), who found that when maize was pollinated with *Tripsacum* pollen, seeds of hybrid origin "adjacent to pure maize seeds are frequently larger and survive longer than hybrid seeds growing alone." If this could happen differentially for the various florets in the present cross, it would be an adequate explanation of the gradation in caryopsis development that is always found, but if it happens more or less simultaneously for all florets, then some other factor or factors such as those quoted for *Phalaris* must also be operative. In any case, it is perfectly clear that a very pronounced difference in the type of caryopsis produced may be found in the absence of any difference in the chromosome numbers of the interacting species.

(b) *Hexaploid with hexaploid*. No crosses involving the combination tetraploid with tetraploid are included in the present series, but the combination hexaploid with hexaploid is represented by the two species pairs—*F. arundinacea* with *F. rubra* and *F. arundinacea* with *F. gigantea*. The cross *F. rubra* with *F. gigantea* has not yet been attempted, so that one side of the possible triangle is missing.

The interaction of hexaploids within the genera *Avena* and *Triticum* has long been studied, and it is well known that the species concerned intercross quite readily and give rise to fertile progeny. The results given by Popova (1929 a) however suggest, although the numbers involved are very small, that *Aegilops juvenalis* and *Ae. crassa* cannot very easily be intercrossed¹. The F_1 hybrids obtained differed in pollen fertility according to the direction of the cross, but both hybrids failed to set seed (presumably from self-pollination).

Popova (1929 b) has also reported an F_1 hybrid from a cross in which

¹ It is possible that low seed setting is, however, due to difficulties in manipulation.

Aegilops juvenalis was the female and *Triticum vulgare* the male parent. The F_1 hybrid is stated to be completely sterile, but the reciprocal cross has apparently not been attempted. Percival (1921) also refers to a hybrid between *Aegilops triaristata* and *Triticum vulgare*.

The *Nicotiana* species having the gametic chromosome number 24 (Christoff, 1928) may, for present purposes, be regarded as hexaploids. We then find that the results obtainable when such species are intercrossed vary considerably. None of the five combinations described has given mature hybrids from both crosses of a pair, and from only three crosses out of eleven were such mature hybrids produced. Further, only in one combination out of five were the results classifiably similar when the cross was made in either direction. The extreme divergence is found in the combination *N. rustica* with *N. Tabacum*. When the former was used as the female parent, mature F_1 hybrids were obtained, but the pollen tubes of *N. rustica* failed to reach the ovaries of *N. Tabacum*.

Even apart from these results for *Nicotiana*, it is clear from those obtained for *Aegilops* species, and for the crosses between *Aegilops* and *Triticum*, that a similarity in chromosome number, even when these are high, does not necessarily lead to high compatibility, although the results for *Avena* and *Triticum* might suggest otherwise.

The combination *F. arundinacea* with *F. rubra* affords a further illustration of this fact. These two species are nominally members of the same genus, but it is evident that the relationship is less close than that existing in the *Avena* and *Triticum* hexaploid species.

Reciprocal crosses gave results which differ materially in certain respects. It is even probable that the higher rate of seed setting resulting from the use of *F. arundinacea* as the female parent is in this instance significant, but even apart from this, the caryopses were quite definitely better developed when the cross was made in this direction. At their best these caryopses were nearly equal in length to those normally produced by the mother plant, while at the same time they were plump in proportion. Such caryopses when produced by intraspecific fertilisation would be expected to germinate strongly, but all these failed to do so. On the other hand, the caryopses produced by *F. rubra* as the female, while of fairly good length at their best, were in all cases very narrow and more or less shrivelled, showing much poorer development than those of the reciprocal cross. Yet some of these were found to be capable of germination, and, by careful nursing, established plants were produced.

These results, particularly in relation to caryopsis type, are unusual, since it is generally found, particularly in wheat (Watkins, 1927, 1932;

Thompson and Cameron, 1928) and also in oats (Kihara and Nishiyama, 1932), that when the type of caryopsis differs in accordance with the direction of the cross, the long, slender and shrivelled caryopses germinate less satisfactorily than those which are shorter but more plump. The difference is usually associated with a difference in the chromosome numbers of the species intercrossed. In the present combination, both species were hexaploids, but there was a definite difference in caryopsis development. In this particular therefore the present results agree with those obtained in the diploid-diploid combination already discussed. Unfortunately, however, the seeds which failed to germinate have not been closely examined, so that relative endosperm development can only be judged by the appearance of the caryopses.

Judging by the appearance of the caryopses, endosperm development was very much better when *F. arundinacea* was used as the female parent. Since these seeds all failed to show any sign of germination, it may probably be assumed that failure in this case was due to the absence of viable embryos¹. That this is not entirely due to the constitution of the embryo itself is proved by the fact that established plants, however weak, have been obtained from the reciprocal cross, although in that case, judging by the appearance of the caryopses, endosperm development is much poorer.

In the other hexaploid-hexaploid combination, *F. arundinacea* with *F. gigantea*, the results were entirely different. Established F_1 hybrids were now obtained without serious difficulty when the cross was made in either direction. Still, a few caryopses which had failed to reach full normal development suggest that development was not quite normal, particularly since these failed to germinate, whereas equally developed seed produced intraspecifically would be expected to germinate quite strongly. Since relatively poor caryopsis development and failure to germinate are in this case associated, it seems probable that the death of the embryo and the arrest of development in the caryopsis as a whole were more or less coincident. That the cross is not of intervarietal status is also proved by the male sterility of the hybrids, but the morphology of the parent plants themselves also entitles each to full specific rank.

From three combinations in which the chromosome numbers of the paired species are similar, therefore, three distinct types of results have

¹ Such an assumption is probably fully justified, since, with artificial incubation, it is possible to observe even the slightest signs of germination, whereas when seeds are planted directly in soil it is necessary that the plumule should develop quite considerably in order that germination may become visible.

been obtained. In one case, diploid with diploid, the cross has been successfully made in both directions but only with the greatest difficulty, and the type of caryopsis produced differed fundamentally according to the direction of the cross. In the hexaploid-hexaploid combination, *F. arundinacea* with *F. rubra*, there was also a difference in caryopsis development when the cross was made reciprocally. In this case the better developed caryopses failed to germinate, while established plants were obtained from the poorer seed of the reciprocal cross. Finally, in the other hexaploid-hexaploid combination, the results were almost comparable with those obtainable from intraspecific crossing. It is therefore evident that factors other than a dissimilarity in chromosome numbers may seriously affect the results obtainable in interspecific and intergeneric crosses.

(c) *Diploid with tetraploid*. The literature concerning this type of interspecific or intergeneric combination is relatively extensive, but since reciprocal crosses have not been made in any of the present combinations, only a very brief review of the results is required.

None of the four combinations described gave fully positive results. The nearest approach to this was the development of some quite good caryopses in the florets of *L. perenne* ($2n = 14$) when pollinated by *F. ovina* ($2n = 28$), though failure to germinate shows that this cross cannot easily be made in the direction indicated. It should be pointed out, however, that the better developed caryopses were not of the extremely long, shrivelled type generally associated with the use of the species with the lower chromosome number as the female parent, but were relatively plump in proportion to their length. Even then, however, it is quite conceivable that better results can be obtained when the cross is made in the opposite direction. It is, in any case, interesting to find that two species, so different both morphologically and ecologically, have shown some definite degree of compatibility.

Another of these combinations, *Glyceria fluitans* with *L. perenne*, is of interest in relation to the origin of the supposed natural hybrid *F. loliacea*. The completely negative results obtained when *Glyceria fluitans* was employed as the female parent suggests no close affinity between the two species, but it is yet possible that the reciprocal cross, when made, may give entirely different results.

The remaining crosses—*L. perenne* ♀ × *Dactylis glomerata* ♂ and *L. perenne* ♀ × *Arrhenatherum avenaceum* ♂—were not expected to give germinable seed, and no sign of ovary stimulation followed pollination.

These two results, coupled with that obtained from *Glyceria fluitans*

$\times L. perenne$, however, show that pollination alone in these cases was insufficient to induce a development of the ovary.

(d) *Diploid with hexaploid*. The best known example of this type of combination in the Gramineae that has yielded mature F_1 hybrids is that of *Triticum vulgare* with *Secale cereale*. Supposed naturally produced hybrids between these two species have frequently been reported. Artificial hybridisation has shown that the cross hexaploid wheat $\text{♀} \times S. cereale$ ♂ can be made with relative ease, especially if particular varieties of *Triticum vulgare* are used (Backhouse, 1916). The general failure of the reciprocal cross led to the expressed opinion that apparently the hybrid cannot be produced if rye is used as the female parent. The claims of Gaines and Stevenson (1922) that they obtained such hybrids have been disputed, particularly by Meister and Tjumjakoff (1928), who found that when they used a particular wheat variety, rye could be used as the female parent with relative ease and that the F_1 hybrids, contrary to the findings of Gaines and Stevenson, did not differ in type according to the direction of the cross. Bledsoe (1932) also reports the production of a single rye-wheat hybrid plant.

Tschermak (1914) states that he was able to cross *Triticum monococcum* with all the wheat forms including those of the Dinkel group, but Melburn and Thompson (1927) consider that apart from this example only one other case was known where the diploid-hexaploid wheat cross had been successfully made until they obtained a strong F_1 hybrid from the cross einkorn \times spelt. Thompson (1930) further states that "the cross between 21- and 7-chromosome wheats is difficult in either direction but distinctly easier when the species with the larger number is used as the female." This statement seems to be supported by the results described by Kihara, Wakakua and Nishiyama (1929), for *Triticum spelta* with *T. aegilopoides* and with *T. monococcum*.

Nishiyama (1929) failed to obtain germinable seed by intercrossing diploid and hexaploid *Avena* species, although in every case there was some measure of caryopsis development. Kihara and Nishiyama (1932) report similar results where the diploid was used as the female parent, but they succeeded in two crosses where the hexaploid species was used in this capacity¹. The results for one of these combinations, *A. strigosa* with *A. fatua*, have already been alluded to above, since they appeared

¹ They also failed to obtain germinable seed in diploid-tetraploid combinations when the former were used as the female parents, whereas in the reciprocal crosses seed germination ranged from 72-73 to 100 per cent. In these, as in the diploid-hexaploid series, seed setting was appreciably higher when the *diploid* served as the female parent.

to be very similar to those obtained in the diploid-diploid combination, *L. perenne* with *F. pratensis*, of the present series.

Certain *Nicotiana* species have the gametic chromosome number 24. If we regard these as hexaploids corresponding to diploids in which the corresponding number is 8, we find that in all the intergroup crosses of this type made by Christoff (1928) mature hybrids were obtained only when the species with the higher chromosome number was used as the female parent.

As far as germinable seeds are concerned, all the above results are in accord with the general conclusion arrived at by Thompson (1930) and by Watkins (1932) that as a rule better results are obtained when the species with the higher chromosome number is used as the female parent¹. It is admitted, of course, that exceptions occur, and some at any rate of the present results must be added to this list of exceptions.

One species pair of the diploid-hexaploid type has so far only been intercrossed in one direction. When *F. pratensis* was used as the female parent with *F. gigantea*, an estimated total germination of 42.9 per cent. was obtained, and six out of eight seedlings survived to become established plants. Since the cross has not been tried in the opposite direction, it is not known whether in this combination a result more closely approaching perfection may be obtainable, but the present result is certainly satisfactory and definitely more so than the similar *Avena* combinations recorded by Kihara and Nishiyama (*loc. cit.*) and the *Nicotiana* combinations of Christoff (*loc. cit.*). It may be recalled also that caryopsis development appeared to be perfect in some seeds despite the use of the diploid as the female parent.

For present purposes the fact that octoploid *F. rubra* plants were to some extent employed in the combination *F. pratensis* with *F. rubra* may be ignored, since there is no evidence that this had any material effect upon the general results. Ovary stimulation and a measure of caryopsis development followed pollination when the cross was made in either direction. Although no germinable seeds were obtained in either case, the better developed caryopses (in relation to the normal caryopses of the mother plants) were produced when the diploid *F. pratensis* served as the female parent. In the complete absence of germination this may not be significant, since other results in the present series show that the highest germination is not always given by the best developed caryopses.

¹ These authors, and also Kihara and Nishiyama (1932), have reviewed the literature in great detail, and have included combinations which are only briefly referred to in the present paper.

The data for the reciprocal crosses between *L. perenne* and *F. rubra* are reasonably extensive and show that seed setting was greatly depressed when the hexaploid was used as the female parent. In this respect the results agree with those for the *Avena* crosses referred to above. It is possible indeed that owing to this the 13 seeds obtained when *F. rubra* served as the female parent were insufficient to be considered representative, but at least two of them should have germinated if seed of equal or better germinating capacity are in this case obtainable when the hexaploid is used as the female parent. In any case, germinable seed and established plants have, so far, only been obtained when the diploid was used as the female parent.

Some of the caryopses produced by the cross *L. perenne* ♀ × *F. rubra* ♂ were particularly well developed while development was generally good with no marked shrivelling. Germination, therefore, at 18.2 per cent. was very low in relation to caryopsis development. When the hexaploid species was used as the female parent, not only were the caryopses very much smaller in size, but they also showed pronounced wrinkling. In two respects therefore the results for this species pair depart from the general rule: (1) the larger and plumper caryopses were produced when the diploid served as the female parent; (2) germinable seeds were obtained also only when the cross was made in this direction.

It is interesting to note that both caryopsis development and germination were better when *L. perenne* was pollinated with the pollen from the hexaploid *F. rubra* than when similarly pollinated with pollen from the diploid *F. pratensis*, while *L. perenne* gave better results than *F. pratensis* in combination with *F. rubra*.

The interaction of the two hexaploids, *F. arundinacea* and *F. rubra*, when paired has already been discussed. *F. arundinacea* ($2n = 42$) and *L. perenne* ($2n = 14$) have also been crossed reciprocally (Cross VII). The caryopses were decidedly better developed when the hexaploid was used as the female parent, but none of the seeds germinated although the number of seeds tested (112) was by no means low. With the diploid as the female parent, there was really no great difficulty in obtaining established plants, although the caryopses were definitely less well developed.

Similarly, in the combination *F. pratensis* with *F. arundinacea*, the diploid served well as the pistillate parent; but in this case the hexaploid *F. arundinacea* served equally well in this capacity. The only significant difference in the results for the reciprocal crosses in this combination is the somewhat better development of the caryopses when the diploid *F. pratensis* was used as the female parent.

The results for the various combinations of diploids with hexaploids therefore vary greatly. In no case was there complete incompatibility in that the ovaries were not stimulated, but no germinable seed was given by *F. pratensis* and *F. rubra* in reciprocal crosses. *F. pratensis* as female with *F. gigantea*, however, gave fully positive results, as also did *L. perenne* with both *F. rubra* and *F. arundinacea*; but from neither of these two crosses has germinable seed been obtained by using the hexaploid as the female parent. The climax is reached with the combination *F. pratensis* with *F. arundinacea*, where the results were very similar throughout whether the diploid or the hexaploid was used as the female parent, and the only difference that may be significant in this case is the somewhat better development of the caryopses in the diploid mother plant.

In the two combinations that have yielded germinable seed only when the cross was made in one direction, the female parent was the diploid. In one of these, the better developed caryopses were also obtained when the cross was made in this direction, but in the other combination the better seed failed to germinate. The fact that the diploid *F. pratensis* gave good results when pollinated with *F. gigantea* is also worthy of note, although the cross has not yet been attempted in the opposite direction.

The present results in the combinations high and low chromosome numbers therefore fail to agree almost at every point with those usually obtained in such cases. Not only are they diametrically opposite in two combinations, but in at least one other, *F. pratensis* with *F. arundinacea*, they are just as different, inasmuch as here the direction in which the cross is made appears to have no appreciable effect upon the result.

(e) *Tetraploid with hexaploid* (?). The literature is probably more extensive with regard to this type of combination than any other, but the present results cannot be discussed in any great detail for two reasons: (1) In the only combination of this type that has been investigated pollination was incomplete when either plant was used as the female parent. It was more complete, however, where the hexaploid was used in this capacity. (2) The *F. rubra* plants have not been investigated cytologically and therefore their chromosome numbers are not definitely known. In all probability they are of the hexaploid type, but the possibility that they may be octoploids cannot be ruled out.

As far as the results go they suggest that the species with the lower chromosome number, *F. ovina*, serves better as the pistillate parent, for from this cross established plants have been secured.

A consideration of the results discussed above shows that: (1) A

difference in the chromosome numbers between two species does not of necessity lead to a very marked difference in the result according to the direction in which they are intercrossed. (2) Species with similar chromosome numbers when intercrossed may give entirely different results according to the direction of the cross, or they may give results which are closely alike. (3) Differences in caryopsis development may be quite as pronounced when species with similar chromosome numbers are intercrossed as when the interacting species are different in chromosome numbers. (4) In the present series, where species differing in chromosome number have been reciprocally intercrossed, and germinating seeds have been obtained only when the cross was made in one direction, the mother plant in all cases has the *lower* chromosome number. (5) In some cases, better caryopsis development has followed the use of the species with the higher chromosome number as the female parent, but such seeds have failed to germinate.

These facts lead us to the conclusion that differences in chromosome numbers, at any rate where the interacting species have the same basic chromosome number, do not entirely determine the degree of compatibility that exists between different species pairs. Thus we find three different types of results where the interacting species are similar in chromosome numbers. Species pairs differing in the same way in so far as chromosome numbers are concerned also give different results. This appears to mean that physiological harmony, as suggested by Müntzing (1930), may be of equal importance, and that a particular cross may or may not succeed irrespective of the chromosome numbers of the interacting species. The success of *L. perenne* in various crosses, particularly when used as the female parent, suggests that this species is physiologically very tolerant.

(4) *The results in relation to phylogeny.*

If compatibility, including a capacity for ovary stimulation and a measure of caryopsis development as the result of interpollination, may be regarded as evidence of phylogenetic relationship, the present results show that in the species employed in the present series of experiments the following are so related: *L. temulentum* with *L. perenne*; *L. perenne* with *F. ovina* (diploid type), *F. rubra* (hexaploid type), *F. pratensis* and *F. arundinacea*; *F. pratensis* with *F. rubra* (hexaploid and octoploid types); *F. arundinacea* and *F. gigantea*; *F. ovina* with *F. rubra* (hexaploid type?); *F. arundinacea* with *F. rubra* and *F. gigantea*. In fact, in all the crosses involving *Lolium* and *Festuca* species that have yet been studied, ovary

stimulation followed pollination, but several possible combinations of the species named above have not yet been attempted.

The genus *Lolium* consists of relatively few species, and all those that have hitherto been examined cytologically have been found to be diploids (Evans, 1926; Faworski, 1927). This suggests two possibilities. Either (1) the genus is of relatively recent origin or (2) fundamental changes sufficient to give rise to taxonomic species are very infrequent in the genus. It is, of course, assumed that all the *Lolium* species are of monophyletic origin, although this remains to be proved. The only pair so far tested for compatibility are *L. temulentum* with *L. perenne*. The results were positive in so far as caryopsis development is concerned, but no germinable seeds have so far been obtained. On the assumption made above, these two species are phylogenetically related. Since they are both diploids, their separation, involving pronounced morphological and physiological characteristics, has been brought about by changes other than chromosome duplication.

While fundamental changes of this type have not been numerous, as judged by the number of extant species, the species *L. perenne* has been particularly successful and is at present one of the most important herbage grass species. Its wide distribution is undoubtedly in part due to the intervention of man, either consciously or unconsciously, but its own "adaptability" is mainly responsible. This "adaptability" consists of a remarkably wide range of types (Jenkin, 1930), all of which, as far as is at present known, are intercrossable, and many very diverse types have actually been intercrossed by the present writer. The changes responsible for the production of this wide variety of types are all presumably of the nature of gene mutations, so that within the genus we find a very conservative element concerning major and fundamental changes, but a great tendency at least in one species towards gene mutations. This latter feature is further illustrated by the fact that of the individual unrelated plants that have yet given seedlings from self-pollination, about 33 per cent. have been found to be heterozygous for factors concerned with some type of defective seedling (Jenkin, 1931 *b*). Only two cases are yet known where two unrelated plants have been found to be heterozygous for the same factor pair concerned with such defects.

The position of the *Lolium* type which I have preferred to regard as *L. perenne* var. *multiflorum*, but which has also been given full specific rank as *L. italicum* A. Br. and as *L. multiflorum* Lam. is difficult to determine. My own results (Jenkin, 1931 *c*) show that it interbreeds quite readily with typical *L. perenne* plants, and the F_1 progeny of the cross

appear to be of normal intraspecific fertility. In F_2 a wide variety of types appears, but amongst them it is difficult to find individuals which may be regarded as pure *L. perenne* or pure *multiflorum*. In fact, the population is again reminiscent of populations obtainable from intercrossing extreme types within a species. At the same time, some of the characters of the *multiflorum* type are strongly suggestive of a possible *L. temulentum* influence, and although the present attempt to intercross *L. temulentum* and *L. perenne* failed to give F_1 hybrids, this result cannot be regarded as conclusive. If such F_1 hybrids can be produced or can occur in nature, then by analogy with results obtained for *Phalaris* species (Jenkin and Sethi, 1932) in later generations, plants more or less similar to the present *multiflorum* might be expected. In any case, whatever the exact derivation of this type may be, it has again been attained without any change in chromosome numbers (Evans, 1926).

Since the *multiflorum* type is also very successful (more especially owing to the activities of man), we find in this genus that polyploidy is not by any means essential to success, and that a species may be able to meet all normal demands upon it by means of other changes.

The position in the genus *Festuca* is very different. Here, somatic chromosome numbers of 14, 21, 28, 42, 56 and 70 have been recorded (Evans, 1926; Levitsky and Kuzmina, 1927; Stählin, 1929; Turesson, 1931; Avdulow, 1931). Taxonomically, even excluding the *Vulpia* group, Hackel (1882) has arranged the species in six sections, with only two of which, the *Ovinæ* (fine-leaved fescues) and the *Bovinae* (broad-leaved fescues), we are at present concerned. All the above chromosome numbers have already been recorded for the *Ovinæ* group, but an octoploid has not yet been reported for the *Bovinae*.

This elaboration of the genus suggests that the genus as such is of very remote origin if we assume a common basis for all types, or even if we assume that they have been in part derived from a common ancestor. Yet, judging from the cytological data available in the literature, a very considerable number of distinct diploid types still exist, so that the polyploid types have probably contributed more to the extension and distribution of the genus than to effective competition in those areas where the diploids existed. One of these diploids, *F. pratensis*, must still be regarded as a very successful type, although probably less so than *L. perenne*. The other diploids (apart from annuals of the *Vulpia* group) appear to have a more limited distribution, but some of them, e.g. var. *capillata* of *F. ovina*, are quite successful in certain environments.

The impression gained in dealing with this genus is, that the fine-

leaved group (*Ovinae*) is based upon some such type as the *capillata* referred to, and the broad-leaved group (*Bovinae*) on *F. pratensis* or its progenitor. But if we include *Festuca* (= *Vulpia*) *bromoides* as a third diploid (Stählin, 1929) the position becomes almost bewildering, since it is difficult to see how these three diploids can possibly be derived from a common ancestor except at an extremely remote distance in time. *F. bromoides* must be dismissed from present consideration since we have no evidence that it is at all compatible with any of the species at present under discussion. Neither has *F. ovina* var. *capillata* been included in the present experiments, but the tetraploid *F. ovina* type used in two combinations is in general so similar to *capillata* that the direct derivation of the tetraploid from the diploid appears to be quite possible. The combination *F. pratensis* with the tetraploid *F. ovina* has, however, not been studied. On the other hand, it has been found that *F. pratensis* and *L. perenne* are sufficiently compatible to give rise to established F_1 hybrids, while *L. perenne* has given more or less well-developed caryopses when intercrossed with the tetraploid *F. ovina*, so that an indirect connection between the latter and *F. pratensis* is established.

The tetraploid *F. ovina* has also been successfully intercrossed with an *F. rubra* type, but the chromosome number of the latter is unknown. In all probability it is a hexaploid, but in any case it is quite easily classifiable as *F. rubra*, and therefore a connection is again established. *F. pratensis* has given caryopses with both hexaploid and octoploid *F. rubra* types, but none of the seed so far obtained has germinated. *L. perenne* has, however, given established plants when used as the female parent with hexaploid *F. rubra*, so that it appears to be more or less related to the three fescue types, *F. pratensis* (diploid), *F. ovina* (tetraploid) and *F. rubra* (hexaploid). In all these cases, however, the relationships appear to be remote, if we judge only by the ease with which F_1 hybrids can be produced.

No attempt has yet been made to intercross any of the broad-leaved fescues with the tetraploid *F. ovina* type, but the hexaploid *F. rubra* has been successfully intercrossed with the hexaploid *F. arundinacea*, although again only with considerable difficulty. Thus a measure of compatibility has been found to exist between *F. rubra* and each of the types *L. perenne*, the tetraploid *F. ovina*, *F. pratensis* and *F. arundinacea*¹.

¹ *F. gigantea* might here be added to the list, since caryopses have been produced from the cross *L. perenne* ♀ × *F. gigantea* ♂. This combination has been omitted from general consideration owing to the fact that the conditions under which the cross was made were very unfavourable. The *multiflorum* type of *Lolium* has been successfully crossed with *F. gigantea* by Nilsson (1930 a).

L. perenne has also been successfully intercrossed with *F. arundinacea*, so that it has given established plants from crosses with three distinct types of fescue, and more or less well-developed caryopses with yet a fourth type.

All the above crosses, however, must be classed as "difficult," although some quite vigorous hybrids have been obtained. None of them, excepting the cross between the diploids *L. perenne* and *F. pratensis*, has given germinable seeds from reciprocal crosses. While therefore a phylogenetic relationship between all these types may probably be inferred, the effect of this common kinship is almost obliterated by changes which have taken place in the subsequent development of the types.

It seems impossible at present to speculate usefully concerning the origin of polyploidy in *F. ovina* and *F. rubra*. Within *F. rubra*, the subspecies *violacea* (Gaud.) Hack. is reported by Stählin (1929) to be a diploid. This particular type is unfamiliar to me, but it should probably rank in date of origin with the diploids *L. perenne*, *F. pratensis* and, say, *F. ovina* var. *capillata*, though since the species *F. ovina* and *F. rubra* are both included in the group *Ovinae*, it may be derived directly from the diploid *F. ovina*¹. No tetraploid *F. rubra* types have yet been reported, but the species has not yet been extensively explored, and in any case the tetraploid form may have been only of passing importance as a means of producing the higher polyploids. So far, however, there is no direct evidence whether the hexaploids and octoploids are derived solely from the diploid *F. rubra*. In *F. ovina* on the other hand, both tetraploids and hexaploids are known, and also a decaploid form, but hitherto no octoploid form. In this species, however, triploids also occur (Turesson, 1931), but it may be doubted whether these are in the direct line of evolution.

When we come to the study of the interaction of the three broad-leaved fescues, we find that the results differ quite materially from those already discussed. The cross between the diploid *F. pratensis* and the hexaploid *F. gigantea* has only been attempted in one direction, but it succeeded without serious difficulty, while the diploid-hexaploid combination *F. pratensis* with *F. arundinacea* has given hybrids with little difficulty when the cross was made in either direction. The hexaploid-hexaploid combination *F. arundinacea* with *F. gigantea* has given yet more fully positive results. Despite wide chromosome number differences therefore we find that certain crosses can be made fairly easily, so that apparently the effect of chromosome difference is overruled by physio-

¹ The reverse is, of course, equally possible.

logical compatibility. The existence of compatibility of a relatively high order therefore suggests that these three types have become separated only in quite recent times phylogenetically, but the separation has resulted in the production of polyploid types which have become established, in the case of *F. arundinacea* somewhat outside, and in that of *F. gigantea* quite definitely outside the areas normally occupied by the diploid *F. pratensis*. Whether *F. arundinacea* and *F. gigantea* are identical in origin or are strictly parallel in this respect cannot be determined, since the morphological and physiological differences in relation to environment by which they are now distinguished may be secondary rather than primary. The ease with which they can be intercrossed with *F. pratensis* suggests that this species (or its progenitor) has in some degree at least entered into both polyploids, but the manner in which it has contributed is obscure. It is curious to note that in these broad-leaved fescues only one type of tetraploid, the variety *Uechtriziana* of *F. arundinacea*, has yet been reported. This variety is apparently of restricted distribution, but it may be a connecting link between *F. pratensis* and the hexaploid types. Here, therefore, we have a case almost parallel with that of *F. rubra*, and it would seem that at least in some cases tetraploids are themselves less important than the diploids from which they have been derived or the polyploids which are derived from them¹. This is, however, otherwise in *F. ovina*, where the tetraploids appear to form a very important group.

It would therefore seem that diploid species, as exemplified particularly by *L. perenne* and *F. pratensis*, may be quite virile and quite able to meet every demand in the way of ordinary competition, and that the main rôle of polyploidy in *Festuca* has been the production of types which are able to flourish and compete under conditions to which the diploids cannot adapt themselves by means of gene mutations. The relative absence of tetraploids in certain groups suggests that these are themselves both incapable of competing with the corresponding diploids and of extending the limits of distribution of the species beyond the diploid limits. That is to say, they are not sufficiently dissimilar from the diploids to compete with the latter nor to colonise new areas. This may also be true of *L. perenne* and, further, that hexaploids, if ever produced, are also in this case unable to succeed in competition. In *F. ovina*, on the other hand, the tetraploid forms are apparently more widely distributed, and in their case the initial change appears to have been more important, since

¹ Ichijima (1926) reports the complete absence of wild tetraploid forms in *Fragaria* while both hexaploids and octoploids are known.

by means of the duplication of the chromosomes the range of the species has apparently been definitely extended. It still remains to be decided whether even in this species the tetraploids enjoy a wider distribution than the diploids on the one hand, or the hexaploids on the other.

SUMMARY.

1. The results obtained from interspecific and intergeneric crosses with herbage grasses in the period 1921-31 are summarised in tabular form and the necessary details are described in the text.

2. Fourteen species pairs have been mated, the crosses in eight of these pairs having been made reciprocally.

3. Definite ovary stimulation followed artificial pollination in eleven of the fourteen pairs.

4. Where reciprocal crosses were made, ovary stimulation occurred when the cross was made in either direction, but the degree of caryopsis development attained or the type of caryopsis produced often differed materially according to the direction of the cross.

5. Altogether eight different species combinations have yielded established F_1 hybrids, some of which have not yet been recorded as occurring in nature while the taxonomic records of the occurrence of others are very few.

6. Crosses between *L. perenne* and *F. pratensis* have given very poor results despite the fact that the supposed natural hybrid between these two species (*F. loliacea* Curt.) has a relatively wide and profuse distribution.

7. The bearing of the present results upon the question of the spontaneous production of hybrids between the species pairs is discussed, particularly in relation to *F. loliacea* Curt.

8. The results are also discussed in relation to taxonomy. It is considered that they do not affect the taxonomic position of the genera *Lolium* and *Festuca*. On the other hand, compatibility tests are regarded as useful for the ultimate classification of species or varieties where morphological characters are ill-defined.

9. The results are further considered in some detail in relation to chromosome numbers.

10. Contrary to the general rule, the greatest measure of success has followed the use of the species with the lower chromosome number as the female parent.

11. A marked difference in the type of caryopsis produced may be

found even when the intercrossed species are similar in chromosome numbers.

12. The results may differ quite materially according to the particular species employed irrespective of their chromosome numbers.

13. Caryopses which differ emphatically in type have been obtained from reciprocal crosses between two diploid species. The difference is descriptively similar to that obtained by other workers from crosses between species differing in chromosome numbers.

14. In one case, the results obtained from reciprocal crosses have not differed significantly (except possibly in degree of caryopsis development) where the interacting species were diploid and hexaploid respectively.

15. Physiological harmony between the species or between the mother plant and the developing embryo and endosperm appears to be of great importance.

16. The results indicate a wide range of affinities as judged by the measure of compatibility shown by the various species pairs, and some degree of phylogenetic relationship is therefore suggested between several different species.

17. The derivation of the *Lolium* and *Festuca* diploid types from a common prototype is supposed to have occurred at a very remote period in time.

18. In the genus *Lolium*, successful further development has been mainly by gene mutation, and in this way the species *L. perenne* has been particularly successful but it has failed to colonise a wide variety of habitats.

19. The genus *Festuca*, on the other hand, although the diploid types are by no means unsuccessful, has been able to extend its territory by the development of polyploid types which flourish under conditions where the diploids fail.

20. The function of polyploidy therefore appears to be the production of types which are capable of extending the ecological range of a genus, while the capacity for relatively frequent gene mutation is to provide "adaptability" within a fixed and limited range of ecological conditions.

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GENETICAL STUDIES IN CULTIVATED APPLES.

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(With Plates IX, X and Twelve Text-figures.)

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INTRODUCTION.

BREEDING experiments and investigations relating to sterility and incompatibility in apples have been in progress at the John Innes Horticultural Institution since the year 1912. Some of the earlier self- and cross-pollinations were made by Mr W. O. Backhouse and Miss I. Sutton. The part of the investigations concerning sterility and incompatibility has been previously reported upon (Crane, 1926; 1931; Crane and Lawrence, 1929, 1930, 1931).

From many of the crosses made very few viable seeds were obtained, and the majority of the seedlings raised from them lacked vigour and made very poor growth. It was subsequently found that this was due to the involved chromosome constitution of the parents, either one or both being triploid with a complement of 51 chromosomes.

A few of the varieties we have used as parents still await cytological examination, but as we have previously shown (Crane and Lawrence,

1930) it is possible to say fairly definitely from fertility, vigour and progeny whether a particular variety is diploid, triploid or aneuploid. We have therefore provisionally included a few unexamined varieties in Tables I and II, as their characters indicate that they are diploids.

INHERITANCE OF FRUIT CHARACTERS.

In Table I are the diploid varieties we have used and a description of the characters of their fruits. The mark in the columns indicates that the varieties possess the characters denoted in the headings. In Table II we have summarised the skin and flesh colour of the individuals which have so far fruited in families raised from crosses between the varieties described in Table I. The results are from records and observations taken over a period of several years. Care is necessary to ensure that the fruits are fully ripe before making final records, as from the time of gathering to maturity changes in colour are frequent, and in particular change in the ground colour from green towards yellow and also changes in the greasiness of the skin are very common.

The range of variation in the families is more or less continuous, therefore although much time and care have been taken in the classification the division into groups is somewhat arbitrary. For this reason, and because the number of individuals in the families is small, it is not possible to attempt a detailed genetic analysis at the present stage of the investigation. Nevertheless, it is evident from the results in Table II that differences in segregation can often be correlated with the characters of the parents, and although there is no complete dominance of any character incomplete dominance is evident in certain cases.

Ground colour.

The ground colour ranges from pale cream through grades of yellow and greenish yellow to green. The character of the ground colour in the progeny is generally associated with the degree and quality of ground colour in the parents. For example, if all the families of more than twenty individuals in which Cox's Orange is a parent are taken and arranged in order in regard to the ground colour of the parents, then the proportion of individuals with green in the ground colour, with the exception of the progeny from Cox and Duchess Favourite, is seen to be related to the amount of green in the parents (see Table III).

Of the twenty-seven families raised from crossing, it is interesting that although deep yellow is the class of highest frequency, only one family, Duchess of Oldenburg \times Golden Spire, gave an F_1 without a trace of

TABLE I.

[illegible]

TABLE II.

Parentage	Flushed			Striped			Ground colour			Flesh colour			Surface													
	Nil	Medium	Deep	Nil	Pale	Medium	Deep	No colour	Flushed only	Striped only	Cream	Yellow		Deep yellow	Greenish yellow	Yellowish green	Pale green	Green	White	Cream	Yellow	Tinged green	Tinged red	Dry	Partially greasy	Greasy
Golden Spire × Cox's Orange	11	30	7	2	25	16	7	2	1	24	10	15	0	2	20	25	3	0	0	9	30	11	6	20	11	19
Northern Greening × Cox's Orange	8	12	31	2	9	4	30	3	1	12	7	34	0	0	0	22	14	18	0	15	29	10	22	23	14	17
Cox's Orange × Lord Derby	3	11	20	8	10	6	10	0	10	3	29	0	0	0	13	11	0	0	0	17	6	3	4	10	10	10
Duchess Favourite × Cox's Orange	20	13	6	1	19	7	11	3	15	4	5	16	0	5	27	11	2	0	0	27	15	6	16	17	8	8
Golden Spire × Beauty of Bath	3	5	11	2	7	6	2	6	1	6	2	12	0	0	7	12	2	0	0	23	15	3	9	17	13	13
Lord Hindlip × Cox's Orange	5	9	12	4	8	6	9	7	2	6	3	19	0	2	6	14	6	2	0	13	14	3	8	7	14	14
Lord Hindlip × Northern Greening	0	10	12	5	3	5	13	6	0	3	0	24	0	1	21	4	1	0	0	13	14	3	19	5	3	3
Cox's Orange × King of Pippins	13	6	2	2	7	11	3	2	6	1	7	9	3	3	9	7	1	0	0	10	10	3	4	9	10	10
Lord Derby × Duchess of Oldenburg	Antonowka × Cox's Orange	9	11	1	15	2	4	1	7	8	2	5	11	2	6	2	1	0	0	10	10	3	3	4	9	10
Antonowka × Cox's Orange	Cox's Orange × Sturmer Pippin	7	15	33	5	16	12	26	6	4	12	3	41	0	1	20	32	5	1	7	27	26	32	44	13	3
Lord Grosvenor × P. Niedzwiedzka	8	3	3	6	4	7	4	5	1	3	7	9	0	2	14	2	1	1	1	11	8	1	10	7	3	11
Royal Jubilee × Northern Greening	9	8	5	0	10	3	7	2	6	4	3	9	0	9	10	2	1	0	0	3	13	6	2	3	10	9
Cox's Orange × Rev. W. Wilks	11	7	4	0	8	4	10	0	6	2	5	9	0	3	15	4	0	0	0	1	14	7	0	2	7	13
Lord Hindlip × Duchess Favourite	3	9	2	3	9	2	6	2	4	0	9	3	5	0	1	8	6	2	0	1	12	4	6	9	4	4
Cox's Orange × Worcester Pearmain	3	2	6	1	2	2	6	2	0	2	3	7	0	0	2	9	0	1	0	2	10	0	2	2	2	5
Cox's Orange × Duchess of Oldenburg	4	5	3	1	3	4	3	3	1	2	3	7	1	1	5	6	0	0	0	3	9	1	3	2	6	5
Lane's Prince Albert × Encore	6	2	4	0	4	1	5	2	2	2	4	4	0	2	4	5	1	0	0	4	7	1	5	0	3	9
Starling Castle × Lord Derby	3	4	0	0	6	1	0	0	2	4	1	0	0	1	3	1	1	1	0	4	2	1	3	2	1	4
King of Pippins × Worcester Pearmain	1	2	2	1	0	5	1	0	0	2	4	1	5	0	2	3	1	0	0	4	2	1	3	4	1	4
Golden Spire × Lord Derby	3	5	0	0	6	2	0	0	3	3	0	2	0	2	5	1	0	0	0	2	6	0	3	4	1	1
Duchess of Oldenburg × Golden Spire	4	3	0	0	3	2	2	0	1	2	3	1	0	2	5	0	0	0	0	3	3	1	2	3	2	2
Cox's Orange × Beauty of Bath	3	1	3	0	3	0	3	1	2	1	3	0	1	5	1	0	0	0	0	3	2	5	1	3	2	2
Cox's Orange × Lane's Prince Albert	5	3	1	1	2	3	2	3	3	1	4	4	0	1	2	6	1	0	0	5	4	1	7	2	2	2
Annie Elizabeth × Lane's Prince Albert	1	4	2	0	2	1	3	1	1	1	4	5	0	1	2	4	0	0	0	6	4	1	7	2	2	2
Cox's Orange × Newton Wonder	2	1	6	0	1	1	5	1	0	1	2	6	0	0	7	2	0	0	0	3	2	2	5	4	5	5
Royal Jubilee × Lane's Prince Albert	7	6	1	0	7	1	5	1	3	4	4	3	1	2	5	6	0	0	0	5	4	7	4	0	4	4
Starling Castle (selfed)	2	3	0	1	2	2	1	1	1	1	1	3	1	1	1	2	1	0	0	3	4	1	1	1	3	2
Antonowka (selfed)	2	4	0	0	3	1	2	0	1	2	1	6	0	2	2	0	0	0	0	3	3	0	1	2	2	2
Cellini Pippin (selfed)	0	2	3	1	0	2	1	3	0	0	2	1	0	1	0	2	0	0	0	3	3	0	1	2	2	1
Rev. W. Wilks (selfed)	2	0	2	0	3	0	1	0	0	0	1	1	0	1	0	4	0	0	1	6	0	0	3	1	3	1

TABLE III.

Parents and ground colours	Percentage of individuals with some degree of green in ground colour
Cox's Orange \times Lord Hindlip (yellowish green)	67
(greenish yellow) \times Sturmer Pippin (yellowish green)	65
" \times Northern Greening (greenish yellow)	59
" \times Duchess Favourite (greenish yellow)	31
" \times Golden Spire (yellow)	56
" \times Lord Derby (yellow)	46
" \times Rev. W. Wilks (cream)	18
" \times Antonowka (cream)	14
" \times King of the Pippins (deep yellow)	15

green in the ground colour, and in this family there were only seven individuals. Antonowka, a variety with a cream ground, when crossed with Cox's Orange (greenish yellow) gave the largest proportion of creams in the F_1 , namely 50 per cent. Lord Derby crossed Duchess of Oldenburg gave three creams in a family of twenty-three individuals, and two other families gave one cream each.

Only one green and six pale greens were recorded in the total of 586 seedlings from crossing. The total number of yellows and yellowish greens are approximately the same.

Anthocyanin colour.

The red anthocyanin colour may occur as an even flush or in stripes. As a flush it may vary in intensity from pale to deep, may extend over the whole fruit or may be confined to the side exposed to the light. Similarly the stripes may occur all over the fruit, be confined to one side, vary from pale to deep in intensity, and in addition vary from a few small flecks through broken stripes of varying dimensions to continuous broad stripes of colour. Both flush and stripes may occur together, independently or not at all. If there is much anthocyanin it is difficult and sometimes impossible to distinguish ground colours which are closely alike, but when fruits with anthocyanin on contrasting grounds are compared, then the difference in fruit colour is found to be very conspicuous. Thus red on cream is a deep "pinkish" colour, on yellow bright red, on greenish yellow brownish red, and on green brown.

The inheritance of anthocyanin irrespective of whether it occurs as a flush or in stripes is evidently highly complex. Of five families raised in which one parent had fruits devoid of anthocyanin, the ratios of pigmented to non-pigmented fruits were approximately 24 : 0, 49 : 1, 2.8 : 1, 2.1 : 1 and 1.7 : 1. In every case there were striped only, flushed only, and striped and flushed individuals. Summing the total numbers of the

different classes in (1) the families in which one parent was without anthocyanin in the fruits, and (2) the remaining nineteen families where both parents were pigmented, the proportion of pigmented to non-pigmented is 4.6 : 1 and 11.3 : 1 respectively.

Analysis of flush and stripe separately suggests that a number of factors must control the inheritance of anthocyanin and its distribution.

Flush. If the nine families in which Cox's Orange was one parent are taken and the combined numbers of the nil and pale compared with the medium and deep classes, then a similar sort of quantitative inheritance is seen as for ground colour, but the results are more irregular. Taking the nil class alone as a percentage of the whole the quantitative inheritance is still seen, though less clearly. It will be observed that only two families from crossing nil with nil (Golden Spire \times Lord Derby) and pale with nil (Stirling Castle \times Lord Derby) gave no medium or deep-flushed individuals, whereas medium \times deep and deep \times deep always segregate pales and nils in addition to mediums and deeps.

Stripe. The inheritance of striping is even more obscure than that of flushing. While it is true that the only families segregating no deep-striped forms were from medium \times nil, pale \times nil and nil \times nil, there is a surprising variety of results, *e.g.* medium \times nil giving deeper forms in the F_1 than medium \times deep and so on. Nevertheless, of the larger families raised from crosses with Cox's Orange the one segregating the greatest number of nil and pale-striped forms was from medium \times nil, and the family with the greatest number of medium and deep individuals from medium \times deep.

Considering these results together it seems probable that (1) ground colour is determined by a number of cumulative factors; (2) yellow ground is partially dominant or epistatic to green ground; (3) the comparatively rare occurrence of forms without anthocyanin suggests that a number of factors are concerned in the inheritance of anthocyanin in the fruit, and it is possible that at least some of the factors are complementary and cumulative. Flush seems to be inherited rather more clearly than striping. The factors determining the inheritance of anthocyanin seem to be dominant, since individuals without anthocyanin give progeny with very little or no anthocyanin, whereas coloured forms crossed together segregate individuals without anthocyanin.

Flesh colour.

Families in which the flesh of one parent is tinged with green give a higher proportion of seedlings with green-tinged flesh than families

where both parents are without a green tinge. Only one family gave no green tinge, *viz.* Cox's Orange \times Rev. W. Wilks. The next highest is Golden Spire \times Beauty of Bath, both without green tinge, which gave 37 without : 3 with green tinge. The greatest proportion of green-tinged individuals is approximately 50 per cent. Since green tinge occurs in the progeny from parents without green tinge the results indicate that complementary factors are involved in the determination of this character. The segregation of cream and yellow flesh is more variable.

In *Pyrus Niedzwetzkyana* the anthocyanin extends to the vegetative parts of the plant, the leaves, flowers, flesh and even the interior of the wood being deeply pigmented, but in the family raised from Lord Grosvenor \times *P. Niedzwetzkyana* the intensity of the anthocyanin in the pigmented seedlings varies.

The red tinge in the flesh of Beauty of Bath does not appear in the crossed progeny raised from it.

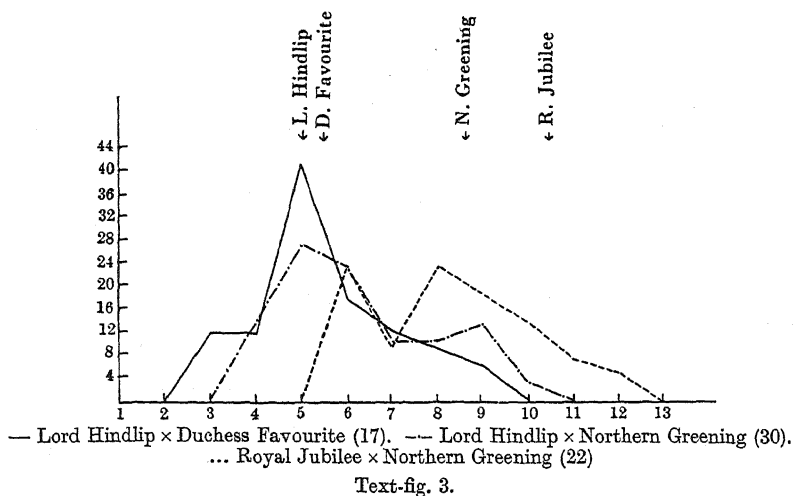
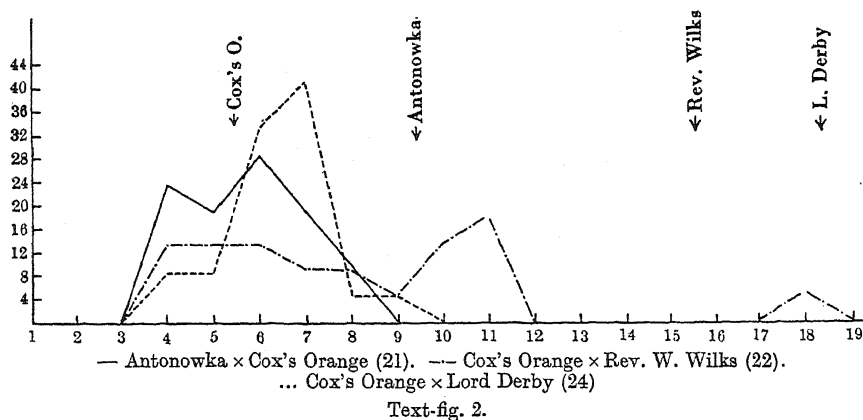
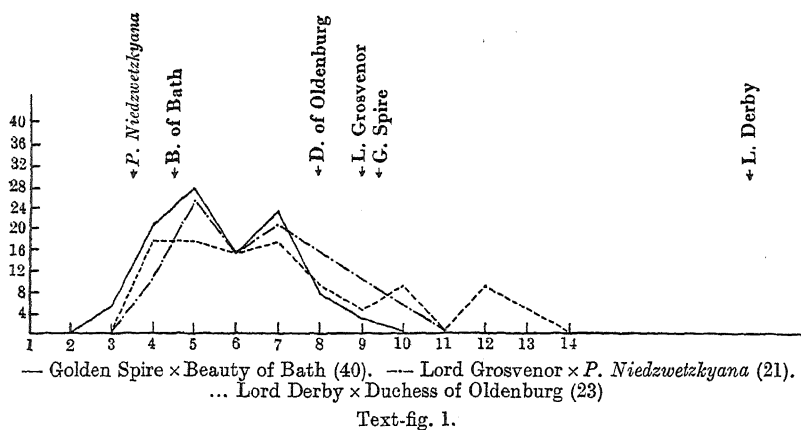
Fruit surface.

Cox's Orange \times Sturmer Pippin (dry \times dry surface) gave 44 dry, 13 partially greasy and 3 greasy, whereas Golden Spire \times Northern Greening, varieties with a greasy surface, when crossed with Cox's Orange give a higher proportion with a partially greasy or greasy surface. Two families from dry \times dry gave 62 per cent. dry. Four families from greasy \times greasy gave 15 per cent. dry. No family failed to segregate greasy and the results indicate that more than one pair of factors is concerned with this character.

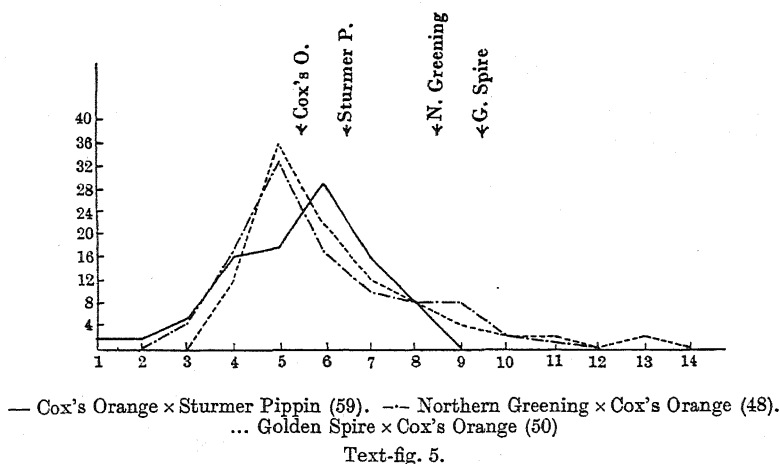
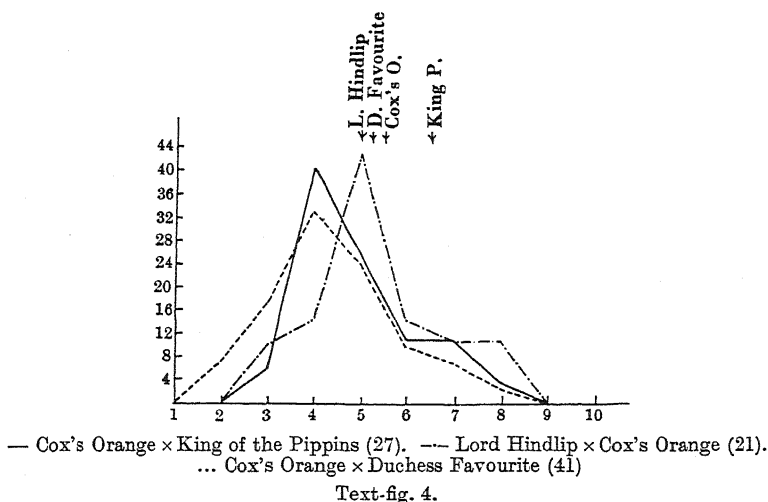
Fruit size.

The size of the fruit of apples is a variable character and fluctuates considerably as a result of differences in environment and other causes. The parental varieties and seedlings, however, have been grown under closely comparable conditions, consequently differences due to these causes are not likely to be considerable. Most of the seedlings dealt with in this report have carried fruits for three or more seasons and their shape and size have been recorded and compared from year to year. Nevertheless, it is impossible accurately to define such variable characters, hence our measurements and determinations must be read as average figures under our conditions.

The range of variation which has occurred in fifteen families with respect to size is presented in Text-figs. 1-5, in which the size of the parents is also shown. The figures denoting fruit size (volume) were



derived from the formula $\frac{\text{length} \times (\text{radius})^2}{1000}$. This product multiplied by 2.7 approximates closely to the actual size of the fruit in cubic centimetres. The mean size of the parents and progeny is given in Table IV.



An analysis of these families with respect to the inheritance of size shows that in three families the mean of the progeny is approximately intermediate between the size of the parents; namely, Cox's Orange x Sturmer Pippin, Lord Grosvenor x *P. Niedzwetzkyana* and Golden Spire x Beauty of Bath. In three families, viz. Antonowka x Cox's

TABLE IV.

Parents	Mean size of parents	Mean size of progeny
Lord Hindlip (4.9) × Duchess Favourite (5.2)	5.05	4.85
Lord Hindlip (4.9) × Cox's Orange (5.3)	5.10	4.77
Cox's Orange (5.3) × Duchess Favourite (5.2)	5.25	3.99
Cox's Orange (5.3) × Sturmer Pippin (6.3)	5.80	5.01
Cox's Orange (5.3) × King of the Pippins (6.7)	6.00	4.13
Lord Hindlip (4.9) × Northern Greening (8.6)	6.75	5.80
Lord Grosvenor (9.1) × <i>P. Niedzwetzkyana</i> (3.6)	6.35	6.05
Northern Greening (8.6) × Cox's Orange (5.3)	6.95	5.31
Golden Spire (9.5) × Beauty of Bath (4.6)	7.05	5.15
Antonowka (9.4) × Cox's Orange (5.3)	7.35	5.21
Golden Spire (9.5) × Cox's Orange (5.3)	7.40	6.04
Royal Jubilee (10.4) × Northern Greening (8.6)	9.50	7.86
Cox's Orange (5.3) × Rev. W. Wilks (15.6)	10.45	7.01
Cox's Orange (5.3) × Lord Derby (18.4)	11.85	5.88
Lord Derby (18.4) × Duchess of Oldenburg (8.0)	13.20	6.67

Orange, Cox's Orange × Lord Derby and Cox's Orange × Rev. W. Wilks, the fruit size in the largest class is slightly larger than that of the smaller parent; and in three families the fruit size in the largest class is approximately the same size as in the smaller parent, viz. Lord Hindlip × Cox's Orange, Lord Hindlip × Duchess Favourite and Lord Hindlip × Northern Greening. In the remaining six families the fruit size in the largest class is smaller than that of the smaller parent.

In all families the mean fruit size of the progeny is smaller than the mean size of its parents. None of the families under discussion has been raised from parents both of which had large fruits, but the progeny showing the largest mean size was obtained from the combination of two comparatively large apples, viz. Royal Jubilee × Northern Greening.

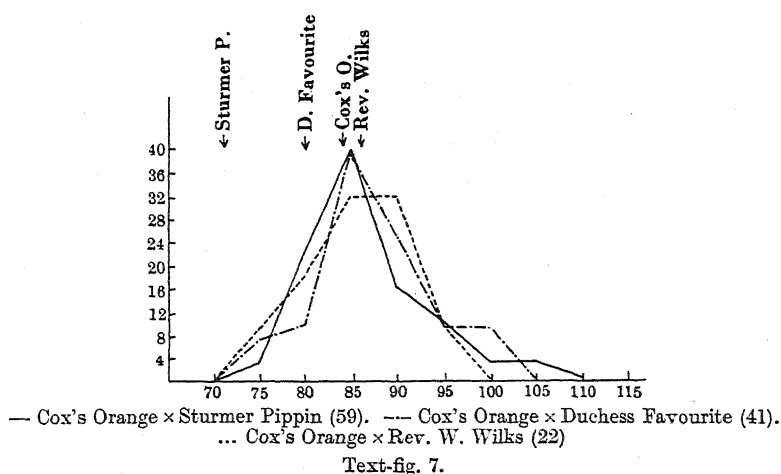
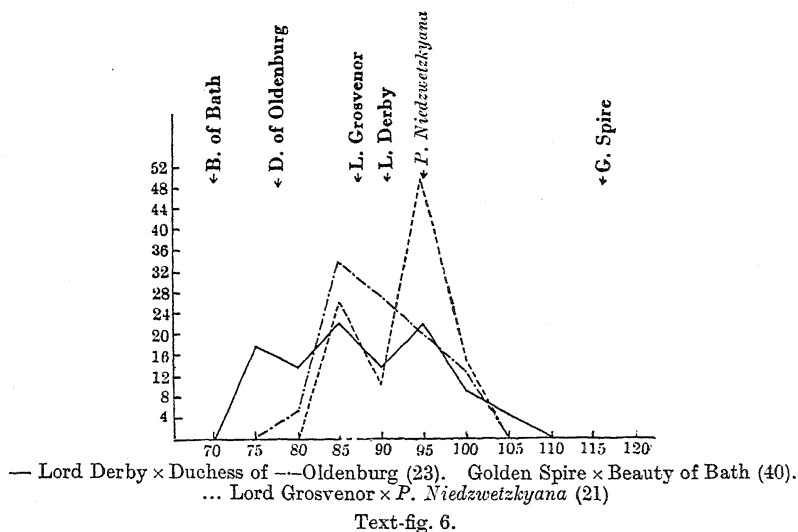
The results suggest that small size is dominant to large size, and that a number of cumulative factors are concerned in the determination of fruit size.

Fruit shape.

Although not such a variable character as size, the shape of the fruit is subject to a certain amount of fluctuation. In some cases this is correlated with the number of seeds formed within the fruit. Fruits with few seeds are frequently more elongated than fruits with many well-developed seeds, but such a correlation is not universal. Our method of recording fruit shape has been to trace the outline of transverse and longitudinal sections of average fruits and to compare them from season to season. Comparison shows that fluctuations are not wide.

The range of variation in a number of families is presented in Text-figs. 6-10, which also give the approximate shape of the parents. The

figures for fruit shape represent the ratio height/breadth expressed as a percentage. At 100 the fruits are practically round, their height and breadth being the same. Above the 100 fruits are long and below 100

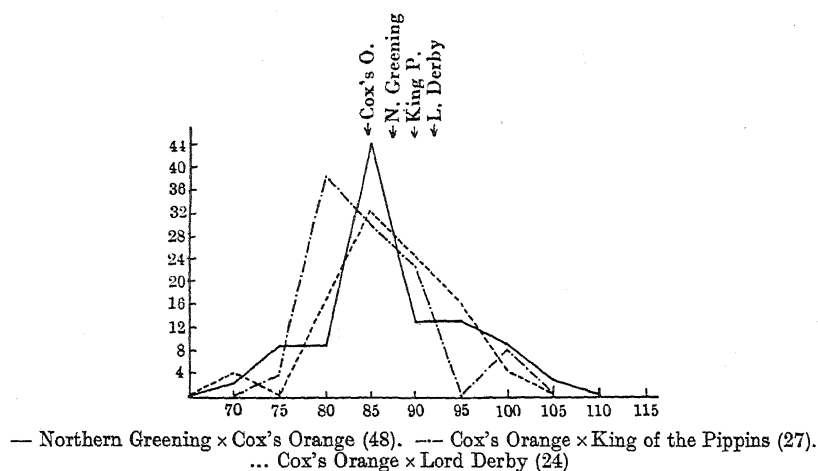


flat. This method of measuring does not take into account obvious finer differences in shape such as the acuteness of the apical and basal parts of the fruit, but at this stage of the investigations no attempt has been made to analyse these finer differences.

The mean shape of the parents and their progeny is given in Table V.

TABLE V.

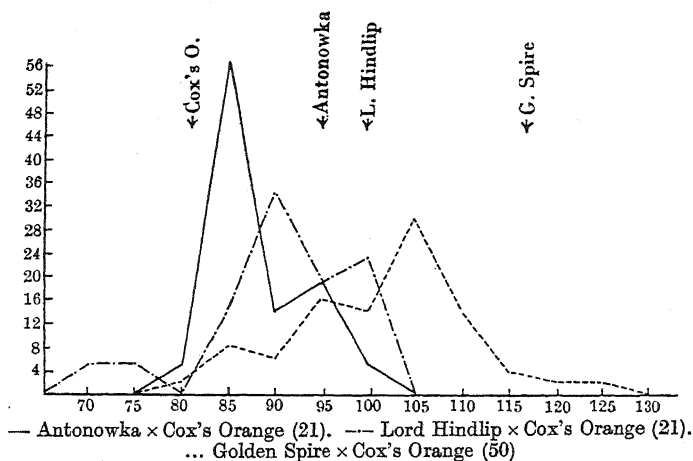
Parents	Mean shape of parents	Mean shape of progeny
Cox's Orange (83) × Sturmer Pippin (73)	78	83
Cox's Orange (83) × Duchess Favourite (80)	81.5	85
Cox's Orange (83) × Rev. W. Wilks (85)	84	84
Northern Greening (85) × Cox's Orange (83)	84	85
Royal Jubilee (85) × Northern Greening (85)	85	89
Lord Derby (92) × Duchess of Oldenburg (78)	85	86
Cox's Orange (83) × King of the Pippins (90)	86.5	83
Cox's Orange (83) × Lord Derby (92)	87.5	85
Antonowka (95) × Cox's Orange (83)	89	86
Lord Grosvenor (83) × <i>P. Niedzwetzkyana</i> (96)	89.5	90
Lord Hindlip (100) × Duchess Favourite (80)	90	88
Lord Hindlip (100) × Cox's Orange (83)	91.5	89
Lord Hindlip (100) × Northern Greening (85)	92.5	90
Golden Spire (118) × Beauty of Bath (70)	94	98
Golden Spire (118) × Cox's Orange (83)	100.5	92



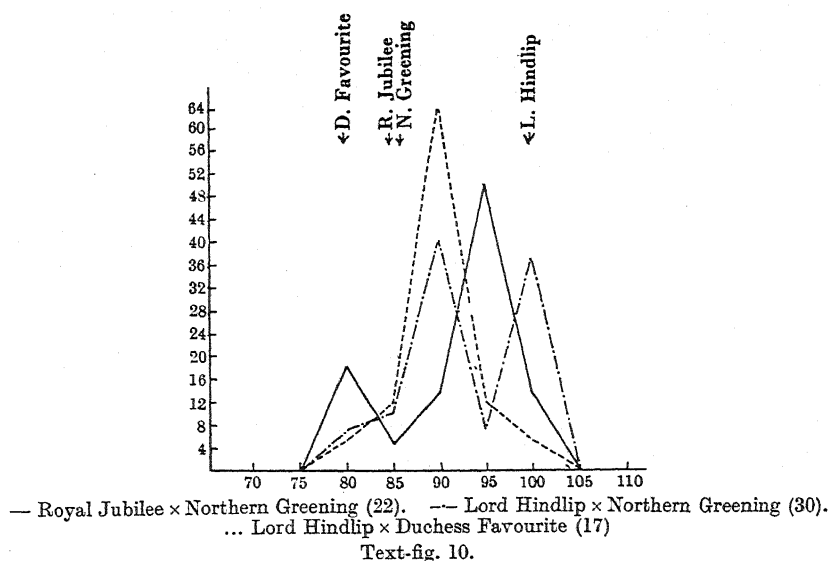
Text-fig. 8.

Although, as is shown in the accompanying graphs, the range of the variation differs, it is evident that there is a certain correlation between the mean shape of progeny and parents. No families have been raised from the extreme flat × flat, or from the extreme tall × tall, but the correlation between the mean shape of parents and progeny is more evident when we consider separately the families in which Cox's Orange, a moderately flat apple, is one of the parents.

Shape	Shape	Mean shape
Cox's Orange 83	× Sturmer Pippin 73	83
"	× Duchess Favourite 80	85
"	× Rev. W. Wilks 85	84
"	× Northern Greening 85	85
"	× King of the Pippins 90	83
"	× Lord Derby 92	85
"	× Antonowka 95	86
"	× Lord Hindlip 100	89
"	× Golden Spire 118	92



Text-fig. 9.



Text-fig. 10.

Fruit flavour.

The flavour of apples varies according to the degree of ripeness of the fruit. Some apples have a fairly high degree of acidity when they first become edible and later their acidity is less pronounced. Owing to this and an almost insensible gradation from one extreme to the other flavour is a particularly difficult character to record. Our method has been to take final records when the fruits are at their best quality and to compare the records from year to year. Quality, however, includes all the flesh characters, such as texture, juiciness and aromatic properties combined, but we have provisionally classified our results according to the degree of sourness or sweetness of the seedlings. In Table VI we have graded the seedlings into five classes from sour to sweet as follows: Sour, briskly sub-acid, sub-acid, slightly sub-acid and sweet.

Time of ripening.

In Table VII we have summarised the season of ripening of the seedlings in the families raised. The number in brackets following the parental varieties is the month of the year in which the fruits reach maturity under our conditions, and the numbers at the head of the table represent the months the seedlings reached maturity. Thus in the family from Golden Spire (10) and Cox's Orange (11-12) four individuals were ripe in the 9th month, 10 at a period covering the end of the 9th and the beginning of the 10th, and 20 individuals in the 10th month, and so on.

No sharply discontinuous variation occurs, but when comparatively early ripening varieties have been intercrossed the time of maturity of the resulting offspring is early. For example, all of the seedlings from Golden Spire (10) and Beauty of Bath (8) ripened between August and October and the majority in August and September. When comparatively late maturing varieties have been intercrossed, the variation although considerable is mainly confined to lateness. Thus the families from Lord Hindlip (2-3) and Northern Greening (12) and Cox's Orange (11-12) \times Sturmer Pippin (3) ripened from November to March. Where families have been raised from comparatively early varieties crossed with later varieties, such as Cox's Orange (11-12) \times Duchess Favourite (9), seedlings as early as the early parent and as late as the late parent occurred, but the majority ripened between the extremes. In some families the variation in the period of ripening exceeded the extremes of the parents, but more often the variation followed that of the parental varieties, being confined to the limits of their extremes.

TABLE VI.

Parents	Sour	Briskly sub-acid	Sub-acid	Slightly sub-acid	Sweet
Golden Spire (sub-acid) × Cox's Orange (slightly sub-acid)	4	10	13	9	14
Northern Greening (sour) × Cox's Orange (slightly sub-acid)	9	10	5	18	7
Cox's Orange (slightly sub-acid) × Lord Derby (sub-acid)	.	3	1	4	16
Duchess Favourite (sub-acid) × Cox's Orange (slightly sub-acid)	.	2	13	15	10
Golden Spire (sub-acid) × Beauty of Bath (slightly sub-acid)	.	3	9	13	16
Lord Hindlip (sub-acid) × Cox's Orange (slightly sub-acid)	.	3	5	4	9
Lord Hindlip (sub-acid) × Northern Greening (sour)	2	8	6	12	2
Cox's Orange (slightly sub-acid) × King of the Pippins (slightly sub-acid)	.	.	6	15	6
Lord Derby (sub-acid) × Duchess of Oldenburg (sub-acid)	.	.	14	3	6
Antonowka (sub-acid) × Cox's Orange (slightly sub-acid)	.	.	6	6	9
Cox's Orange (slightly sub-acid) × Sturmer Pippin (briskly sub-acid)	.	15	15	21	8
Lord Grosvenor (briskly sub-acid) × <i>P. Niedzwiedzkyana</i> (bitter-sweet)	1	3	5	4	7
Royal Jubilee (sub-acid) × Northern Greening (sour)	3	4	7	4	11
Cox's Orange (slightly sub-acid) × Rev. W. Wilks (sub-acid)	.	2	3	6	6
Lord Hindlip (sub-acid) × Duchess Favourite (sub-acid)	.	.	.	8	4
Cox's Orange (slightly sub-acid) × Worcester Pearmain (slightly sub-acid)	.	.	6	3	4
Cox's Orange (slightly sub-acid) × Duchess of Oldenburg (sub-acid)	3	6	3	3	.
Lane's Prince Albert (briskly sub-acid) × Encore (sub-acid)	.	.	4	2	3
Stirling Castle (sub-acid) × Lord Derby (sub-acid)	.	.	2	2	3
King of the Pippins (slightly sub-acid) × Worcester Pearmain (slightly sub-acid)	.	.	6	2	1
Golden Spire (sub-acid) × Lord Derby (sub-acid)	.	.	6	1	5
Duchess of Oldenburg (sub-acid) × Golden Spire (sub-acid)	.	.	.	2	.
Cox's Orange (slightly sub-acid) × Beauty of Bath (slightly sub-acid)	.	5	1	4	.
Cox's Orange (slightly sub-acid) × Lane's Prince Albert (briskly sub-acid)	.	5	1	.	.
Annie Elizabeth (briskly sub-acid) × Lane's Prince Albert (briskly sub-acid)	.	4	6	3	.
Cox's Orange (slightly sub-acid) × Newton Wonder (briskly sub-acid)	4	1	3	3	2
Royal Jubilee (sub-acid) × Lane's Prince Albert (briskly sub-acid)	2	2	2	.	2
Stirling Castle (selfed) (sub-acid)	.	3	2	1	2
Antonowka (selfed) (sub-acid)	.	1	.	1	2
Celini Pippin (selfed) (sub-acid)
Rev. W. Wilks (selfed) (sub-acid)

TABLE VII.

Parentage and season	Season of ripening (month)											
	7th	8th	9th	10th	11th	12th	1st	2nd	3rd	4th		
Golden Spire (10) × Cox's Orange (11-12)	.	.	4	10	20	5	5	2	4	.	.	.
Northern Greening (12) × Cox's Orange (11-12)	.	.	.	1	3	7	7	12	5	3	6	4
Cox's Orange (11-12) × Lord Derby (11)	4	2	5	6	1	5	.	.
Duchess Favourite (9) × Cox's Orange (11-12)	.	1	3	12	9	4	4	6	2	1	.	.
Golden Spire (10) × Beauty of Bath (8)	.	11	16	9	3	1
Lord Hindlip (2-3) × Cox's Orange (11-12)	.	.	.	1	2	6	3	4	3	1	1	1
Lord Hindlip (2-3) × Northern Greening (12)	2	1	8	6	5	1	5	1
Cox's Orange (11-12) × King of Pippins (10-11)	.	.	3	2	10	2	2	5	2	.	.	.
Lord Derby (11) × Duchess of Oldenburg (8)	.	.	4	6	4	6	2	1
Antonowka (10-11) × Cox's Orange (11-12)	.	.	1	4	3	2	3	1	4	2	1	1
Cox's Orange (11-12) × Sturmer Pippin (3)
Lord Grosvenor (9) × <i>P. Niedzwetzkyana</i> (8-9)	.	.	.	3	1	4	2	2	3	6	1	1
Royal Jubilee (10-11) × Northern Greening (12)	.	1	.	.	.	2	2	8	2	.	.	.
Cox's Orange (11-12) × Rev. W. Wilks (9-10)	.	.	.	3	1	7	3	2	.	5	5	3
Lord Hindlip (2-3) × Duchess Favourite (9)	.	.	2	5	4	3	1	.	.	2	.	.
Cox's Orange (11-12) × Worcester Pearmain (9-10)	.	1	.	2	4	1	2	2
Cox's Orange (11-12) × Duchess of Oldenburg (8)	.	1	.	2	1	8	1
Lane's Prince Albert (1-3) × Encore (3-5)	.	.	.	1	1	1	1	.	1	1	1	1
Stirling Castle (9-10) × Lord Derby (11)	.	.	.	1	1	1	3
King of Pippins (10-11) × Worcester Pearmain (9-10)	.	.	.	1	1	1	4	1	2	.	.	.
Golden Spire (10) × Lord Derby (11)	.	.	.	3	1	1
Duchess of Oldenburg (8) × Golden Spire (10)	.	2	.	1	2	1
Cox's Orange (11-12) × Beauty of Bath (8)	.	.	1	2	1
Cox's Orange (11-12) × Lane's Prince Albert (1-3)	2	2	2	3	.	2	.	.
Annie Elizabeth (1-5) × Lane's Prince Albert (1-3)	1	.	.	1	1	3	2	.
Cox's Orange (11-12) × Newton Wonder (12-3)	2	2	1	1	1	2	.	.
Royal Jubilee (10-11) × Lane's Prince Albert (1-3)	.	.	.	2	4	1	1	.	2	1	3	.
Stirling Castle (selfed) (9-10)	.	.	2	1	.	1	1	1
Antonowka (selfed) (10-11)	.	.	.	1	1	1	1	1	1	.	.	.
Cellini Pippin (selfed) (10-11)	.	.	1	.	.	2	1	.	1	1	.	.
Rev. W. Wilks (selfed) (9-10)	.	.	.	1	3

ALBINISM.

Among the families of apples we have raised, a number of albinotic seedlings have occurred. The families in which they have appeared are given in Table VIII.

TABLE VIII.

Parents	No. of seeds	No. germinated	Green	Yellow	Albino	Variegated
1/19 Rev. W. Wilks (selfed)	39	29	25	—	4	—
1/21 Rev. W. Wilks (selfed)	35	31	22	—	9	—
1/22 Rev. W. Wilks (selfed)	25	16	12	—	4	—
6/23 Rev. W. Wilks (selfed)	10	9	7	—	2	—
Total	109	85	66	—	19	—
10/29 Ellison's Orange (selfed)	9	8	7	—	1	—
4/22 Royal Jubilee × Northern Greening	64	45	42	—	—	3
1/23 FrenchParadise (Type VIII) × Jaune de Metz (Type IX)	242	177	134	21	22	—
1/32 Jaune de Metz × Rev. W. Wilks	106	96	90	5	—	—
2/32 Jaune de Metz × French Paradise	30	25	18	5	2	—
3/32 French Paradise × Rev. W. Wilks	17	11	11	—	—	—
4/32 Rev. W. Wilks × French Paradise	7	7	7	—	—	—
5/32 Rev. W. Wilks × Jaune de Metz	15	14	14	—	—	—

The variety Rev. W. Wilks has been selfed on four occasions and has given a total of 66 greens and 19 albinos. French Paradise (Malling Type VIII) × Jaune de Metz (Malling Type IX) gave 134 greens, 21 yellow and 22 albinos, and in a small family raised from the reciprocal cross green, yellow and albino plants also occurred. The albinos all died shortly after germination, and a number of the yellow seedlings a few months after germination. The remaining yellows persisted, and each year when their growth buds burst the leaves were intensely yellow, but as the season advanced they changed to pale green.

Attempts to raise selfed families from French Paradise and Jaune de Metz were not successful. French Paradise developed numerous fruits which proved to be entirely seedless. Very rarely Jaune de Metz when selfed set a fruit with a solitary seed, and the seedlings raised were all green. Crosses were then made between Rev. W. Wilks, French Paradise and Jaune de Metz, as it seemed possible that analysis of such families might assist in elucidating the mode of inheritance of the albinotic forms. In none of these families did albinos appear. Jaune de Metz × Rev. W. Wilks gave 90 green and 5 yellow plants. The seedlings in the other families were all green, but unfortunately only small families were raised, and possibly this may account for no yellows appearing.

The results are insufficient to permit of definite conclusions, but the approximation to a 3 : 1 ratio obtained from selfing Rev. W. Wilks could

be expected for either disomic or polysomic inheritance, *e.g.* either **Aa** or **Aaaa**, etc., will give a 3 : 1 ratio upon selfing. The simplest interpretation of the close approximation to the 6 : 1 : 1 ratio obtained from French Paradise and Jaune de Metz involves two independent factors for green, **A** and **B**, which are epistatic to a third factor **C** for yellow, so that **abc** is white, **abC** yellow and all other types green. Such a ratio could be obtained from crossing two green plants of the constitution **Aabbcc** and **aaBbCc**, but the absence of white seedlings in families 1, 3, 4 and 5/32 shows that this interpretation is not adequate to account for all the results. Since no albinos occurred in these families, presumably those which occurred in the family from selfing Rev. W. Wilks were genetically different from those from French Paradise \times Jaune de Metz.

With the exception of two varieties, all the parents used in Table VIII are known to be diploids, and the two varieties so far unexamined have all the characters of diploids. From a comparison of Tables VIII and XI it is evident that the proportion of non-viable seeds in the families segregating albinos is approximately 100 per cent. greater than in the families giving green plants only. It therefore appears that albinism and a high degree of non-viability are correlated, and possibly certain albinotic genotypes are eliminated.

In families raised from triploid \times diploid, occasional albino plants have appeared. Variegated seedlings occurred in the family raised from Royal Jubilee \times Northern Greening. The variegation in these seedlings was evident in the cotyledons and in all subsequent leaves. In many families, occasional seedlings which were originally wholly green have developed variegated shoots.

ROOT BURRS.

The free formation of root burrs on the stems and branches of cultivated apples is of rare occurrence. It is, however, characteristic of many of the root stocks propagated by layering. Indeed, root burrs (which represent incipient roots) appear to be one of the principal characters which separate the Paradise root stocks from the cultivated apples. In the families we have raised from cultivated varieties, seedlings with varying degrees of burring have occurred. The variation ranges from seedlings which are entirely free from root burrs to seedlings with numerous pronounced burrs, and in Table IX we have classified the seedlings according to the amount of root burrs formed, grade 0 being entirely free, grade 1 only occasional burrs, grade 2 more frequent, up to grade 5 where burrs are abundant and very pronounced.

The majority of the parents of the seedlings in Table IX are entirely free and none has developed root burrs above our grade 1, although the parent trees observed were much older than the seedlings and have been grown under strictly comparable conditions. Considerable variation

TABLE IX.

Parents	Root burrs					
	0	1	2	3	4	5
Golden Spire × Cox's Orange	7	17	11	13	3	1
Northern Greening × Cox's Orange	15	24	10	5	0	0
Cox's Orange × Lord Derby	11	9	2	2	0	0
Duchess Favourite × Cox's Orange	11	16	6	4	1	0
Golden Spire × Beauty of Bath	55	19	7	3	0	0
Lord Hindlip × Cox's Orange	4	4	3	6	2	2
Lord Hindlip × Northern Greening	8	16	12	3	0	0
Cox's Orange × King of the Pippins	11	3	5	3	0	0
Lord Derby × Duchess of Oldenburg	10	10	3	1	1	0
Antonowka × Cox's Orange	5	9	18	6	3	0
Cox's Orange × Sturmer Pippin	62	2	3	0	0	0
Lord Grosvenor × <i>P. Niedzwetzkyana</i>	13	7	0	1	1	0
Royal Jubilee × Northern Greening	43	7	0	0	0	0
Cox's Orange × Rev. W. Wilks	31	2	0	0	0	0
Lord Hindlip × Duchess Favourite	2	6	4	2	4	0
Cox's Orange × Worcester Pearmain	17	3	2	3	0	0
Cox's Orange × Duchess of Oldenburg	3	4	5	2	0	0
Lane's Prince Albert × Encore	13	2	1	2	0	0
Stirling Castle × Lord Derby	6	1	0	0	0	0
King of the Pippins × Worcester Pearmain	4	2	2	0	1	0
Golden Spire × Lord Derby	3	0	2	1	2	0
Duchess of Oldenburg × Golden Spire	2	1	1	0	0	0
Cox's Orange × Beauty of Bath	1	8	6	3	0	0
Cox's Orange × Lane's Prince Albert	7	2	2	2	0	0
Annie Elizabeth × Lane's Prince Albert	0	4	2	3	1	1
Cox's Orange × Newton Wonder	16	1	0	0	0	0
Royal Jubilee × Lane's Prince Albert	32	1	0	0	0	0
Stirling Castle (selfed)	11	0	0	0	0	0
Antonowka (selfed)	5	4	0	4	0	1
Cellini Pippin (selfed)	5	0	0	0	0	0
Rev. W. Wilks (selfed)	35	0	0	1	0	0

occurs in the time the root burrs develop. On some seedlings they appear on one- to two-year-old growth, whilst on others four or more years may elapse before root burrs form on the oldest part of the trees.

In Table X we have summarised the results from families raised from crosses between some of the Paradise apple stocks. Although some

TABLE X.

Parents	Root burrs					
	0	1	2	3	4	5
Doucín × French Paradise	0	8	13	15	49	4
French Paradise × Doucín	0	2	9	5	11	0
Doucín Amélioré (selfed)	0	3	6	3	2	0
French Paradise × Jaune de Metz	11	10	54	36	22	0

variation occurs in the quantity of root burrs which develop, all the parents of these families form root burrs to a considerable extent.

The seedlings in the family raised from French Paradise \times Jaune de Metz were only recorded for two years, as at the end of the second year the seedlings were cut down just above the ground-level and stooled for the production of layers. The seedlings in the other families were observed and recorded over a period of five years. During that time they were grown without pruning or any interference. It seems highly probable that if the family raised from French Paradise \times Jaune de Metz had been grown a longer time without interference that some degree of root burrs would have developed on all the seedlings.

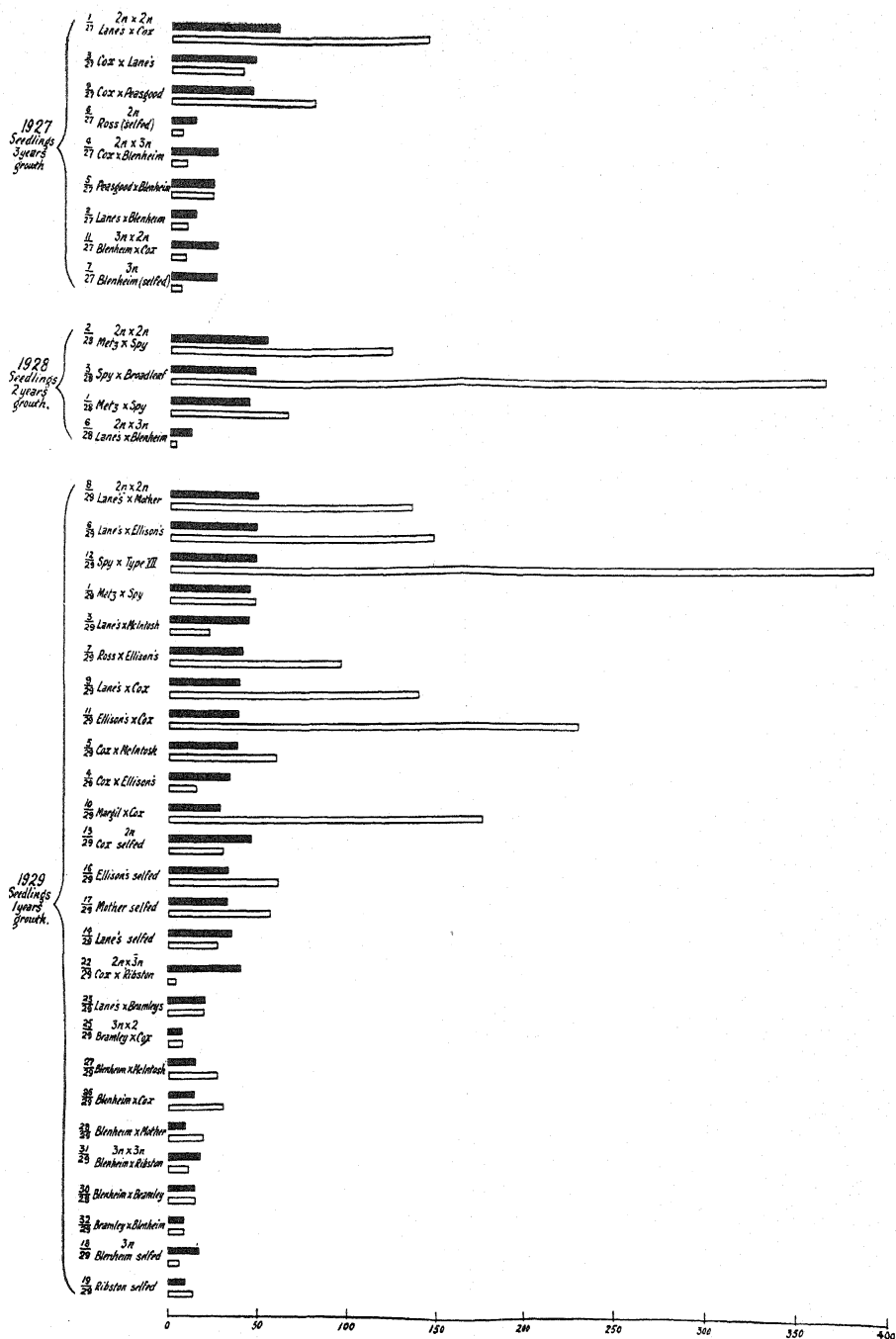
As with the characters we have previously discussed, the degree of burring grades from one extreme to the other. Consequently, although care has been taken in the classification, the groups are somewhat arbitrary and further work is necessary to show if they are genetically distinct. The results, however, suggest that several recessive factors are involved in the determination of root burrs, since (1) they appear in degree in families raised from varieties which are free from root burrs, and (2) parents with a high degree of burring give offspring all possessing some degree of burring. The results also indicate that the factors concerned are cumulative, and as the completely recessive condition is approached the higher is the degree of burring.

Extreme root-burr development has usually been associated with somewhat dwarf growth and fibrous roots, but occasionally seedlings with vigorous growth have had a considerable degree of root-burr development.

In addition to the characters described we have recorded various others, such as the shape of the calyx tube, the position of the stamens, the russet condition of the skin of the fruit, and various characteristics of the carpels, sepals and leaves. The shape of the calyx tube and the position of the stamens grade insensibly from one extreme to the other, and no sharply discontinuous segregation has occurred in respect of the other characters.

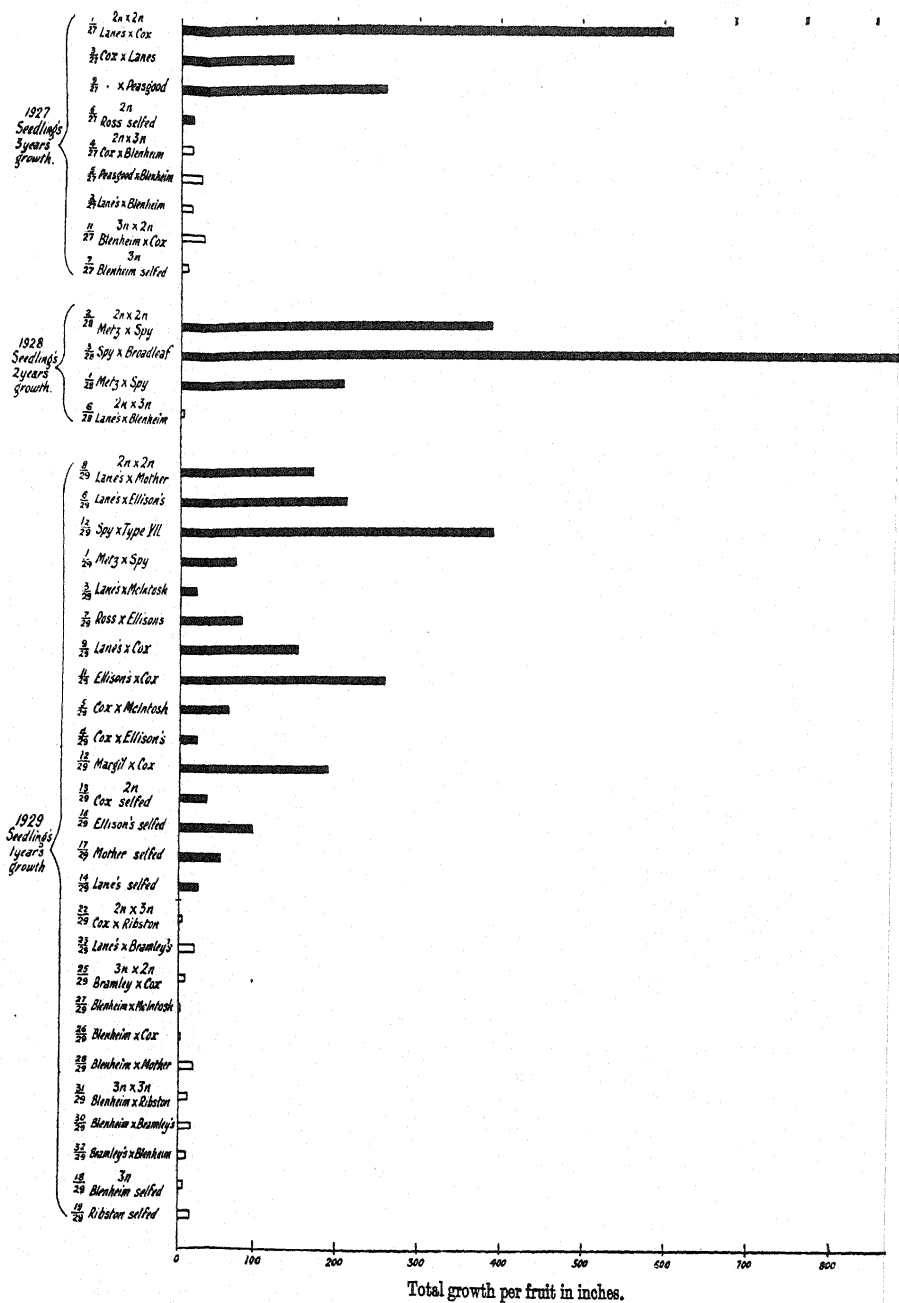
TRIPLOID VARIETIES.

At the time these experiments were begun nothing was known of the chromosome complement of apples. During recent years, however, numerous species and varieties of apples have been cytologically studied by a number of investigators (Rybin, 1926; Kobel, 1927; Crane and Lawrence, 1929; Nebel, 1929 *a, b*, 1930; Darlington and Moffett, 1930;



■ = Average height in inches. □ = Mean gross height of seedlings per fruit.

Text-fig. 11.



Text-fig. 12.

Sax, 1931-2; Heilborn, 1928, 1932; Howlett, 1931-2; Moffett, 1931 *a, b*; Natividade, 1932). These studies have shown that our cultivated varieties of apple consist of two kinds, the so-called diploids and triploids with 34 and 51 chromosomes respectively.

Kobel reported a number of varieties he examined to be aneuploids with intermediate numbers, but as we have previously pointed out (Crane and Lawrence, 1930), a number of the varieties reported by Kobel to be aneuploids have subsequently been found to be triploids and it is doubtful whether any of the established varieties he studied are aneuploids. Many varieties have been investigated, both in Europe and America, and excepting Kobel's report no aneuploid forms have been definitely reported among productive varieties.

Aneuploids arise as a natural consequence of breeding from triploids and exceptional forms may occur, but the vigour and productivity of aneuploids which we have raised at Merton has not so far been sufficiently high to consider their ever becoming widely grown varieties. Therefore although it cannot be definitely stated that aneuploid forms do not occur among the numerous varieties of apples which exist, their general lack of vigour and productivity indicates that they are not likely to occur in cultivation as widely grown varieties.

In an earlier paper (Crane and Lawrence, 1930) we published some preliminary results with apples. In this paper we drew attention to the high degree of generational sterility which occurs among the triploid varieties and to the weak and feeble growth invariably evident in families where either one or both parents were triploids. Since then we have obtained further data from breeding experiments and the results are detailed in Table XI and Text-figs. 11 and 12.

The occurrence of non-viability of seeds and wide variation in the vigour of seedlings in families of apples have been commonly reported upon in pomological literature. Wellington (1924), Crandall (1924), Lantz (1925), Lantz and Edgcombe (1930), Dahl and Johansson (1924), Dahl (1930), Dickson (1928) and many other investigators have described the occurrence of such variations. Owing to lack of knowledge of the chromosome complement of the varieties used as parents it is not possible to consider all the cases to which the above investigators refer, but in many cases it is clear that the lack of vigour and feeble growth they describe is the result of using triploid varieties as parents. Wellington (1924) describes the extreme feebleness of the progeny where Baldwin and Gravenstein have been used as parents; both are triploid varieties. Dahl and Johansson found that Belle de Boskoop and Gravenstein varieties,

TABLE XI.

Family No.	Parentage	Flowers pollinated	Fruits matured	Apparently good seeds	Good seeds per fruit	No. seeds germinated	No. seedlings surviving	Average height of seedlings in inches	Total growth of family in inches
	Diploid × Diploid								
1/27	Lane's Prince Albert × Cox's Orange	87	10	27	2.7	26	24	60.2	6080
3/27	Cox's Orange × Lane's Prince Albert	110	8	10	1.2	8	7	47.0	1163
9/27	Cox's Orange × Peasgood's Nonsuch	89	10	28	2.8	28	17	46.3	2604
6/27	Charles Ross (selfed)	139	2	1	0.5	1	1	14.2	34
	Diploid × Triploid								
4/27	Cox's Orange × Blenheim Orange	160	12	14	1.1	9	4	26.2	170
5/27	Peasgood's Nonsuch × Blenheim Orange	42	2	4	2.0	2	2	24.5	62
2/27	Lane's Prince Albert × Blenheim Orange	18	3	9	3.0	8	2	14.0	44
	Triploid × Diploid								
11/27	Blenheim Orange × Cox's Orange	60	3	12	4.0	5	1	26.0	99
	Triploid (selfed)								
7/27	Blenheim Orange (selfed)	218	5	11	2.2	3	1	25.5	37
	Diploid × Diploid								
2/28	Jaune de Metz × Northern Spy	—	7	22	3.1	18	16	53.9	2730
3/28	Northern Spy × Old English Broadleaf	117	10	84	8.4	82	76	48.0	9940
1/28	Jaune de Metz × Northern Spy	—	4	8	2.0	7	6	44.1	828
	Diploid × Triploid								
4/28	Cox's Orange × Blenheim Orange	36	3	5	1.6	2	0	—	—
6/28	Lane's Prince Albert × Blenheim Orange	109	4	2	0.5	2	1	12	17
	Diploid × Diploid								
8/29	Lane's Prince Albert × Mother	20	4	13	3.2	13	11	49.2	680
6/29	Lane's Prince Albert × Ellison's Orange	42	1	3	3	3	3	49.0	1423
12/29	Northern Spy × Malling Type VII	29	1	8	8	8	8	48.8	391
1/29	Jaune de Metz × Northern Spy	307	11	13	1.1	12	12	45.0	831
3/29	Lane's Prince Albert × McIntosh Red	23	2	1	0.5	1	1	44.0	44
7/29	Charles Ross × Ellison's Orange	23	3	8	2.6	7	7	41.5	350
9/29	Lane's Prince Albert × Cox's Orange	70	5	20	4.0	18	18	39.3	763
11/29	Ellison's Orange × Cox's Orange	121	1	9	9.0	7	6	38.3	259
5/29	Cox's Orange × McIntosh Red	326	21	38	1.8	35	35	38.0	212
4/29	Cox's Orange × Ellison's Orange	336	15	7	0.4	7	7	34.0	335
10/29	Margil × Cox's Orange	50	7	49	7.0	45	44	28.2	1344

TABLE XI (continued).

Family No.	Parentage	Flowers pollinated	Fruits matured	Apparently good seeds	Good seeds per fruit	No. seeds germinated	No. seedlings surviving	Average height of seedlings in inches	Total growth of family in inches
	Diploid (selfed)								
13/29	Cox's Orange (selfed)	1616	13	10	0.7	9	9	46.5	495
16/29	Ellison's Orange (selfed)	229	4	9	2.2	8	7	38.5	379
17/29	Mother (selfed)	284	2	6	2.5	4	3	38.0	114
14/29	Lane's Prince Albert (selfed)	475	9	7	0.7	7	7	35.7	250
15/29	Charles Ross (selfed)	35	1	1	1.0	0	0	—	—
	Diploid × Triploid								
22/29	Cox's Orange × Ribston Pippin	124	9	1	0.1	1	1	40.0	52
23/29	Lane's Prince Albert × Crimson Bramley	57	4	10	2.5	6	4	21.0	84
21/29	Cox's Orange × Blenheim Orange	155	8	0	0	0	0	—	—
20/29	Lane's Prince Albert × Blenheim Orange	38	2	0	0	0	0	—	—
	Triploid × Diploid								
25/29	Crimson Bramley × Cox's Orange	10	1	1	1.0	1	1	8.0	8
27/29	Blenheim Orange × McIntosh Red	59	7	15	2.1	13	13	15.1	207
26/29	Blenheim Orange × Cox's Orange	107	8	17	2.1	17	17	14.5	270
28/29	Blenheim Orange × Mother	59	1	4	4.0	4	2	9.5	19.0
24/29	Blenheim Orange × Ellison's Orange	24	2	1	0.5	1	—	—	—
	Triploid × Triploid								
31/29	Blenheim Orange × Ribston Pippin	35	3	4	1.3	4	2	17.5	35.0
30/29	Blenheim Orange × Crimson Bramley	72	1	2	2.0	1	1	15.0	15.0
32/29	Crimson Bramley × Blenheim Orange	51	1	2	2.0	1	1	9.0	9.0
	Triploid (selfed)								
18/29	Blenheim Orange (selfed)	339	8	12	1.5	3	3	17.3	52.0
19/29	Ribston Pippin (selfed)	123	8	21	2.5	21	13	9.1	119.0

of vigorous growth, in general gave very weak offspring, whereas Reinette Ananas and Bismarck, although themselves of less vigorous growth than Belle de Boskoop and Gravenstein, gave strong vigorous seedlings. These authors suggested that there was a correlation between weight of seeds and the vigour of seedlings, as the seeds from Belle de Boskoop and Gravenstein were lighter in weight than those of Reinette Ananas and Bismarck. Belle de Boskoop and Gravenstein are triploids, and Reinette Ananas and Bismarck, although unexamined, have all the characters of diploids. Dickson (1928) from the results of his investigations suggested that "with age of variety, abnormalities occur which cause low seed content, poor germination of seed and lack of vigour in the seedlings." Among the varieties which Dickson investigated are the triploid varieties Belle de Boskoop, Baldwin, Blenheim, Gravenstein, Ribston and Stayman. It is therefore evident that the correlation between weight of seeds and vigour of seedlings suggested by Dahl and Johansson, and age and sexual degeneracy suggested by Dickson, are the natural consequences which arise from using triploid varieties as parents. The imperfections of the seeds and the lack of vigour of the seedlings inevitably result from the irregularities in germ-cell formation in the parents, and the aneuploid chromosome constitution of their offspring.

The offspring from selfing diploid varieties are often weak, and considerable variation in the viability of the seeds and in the vigour of the seedlings occurs within families raised from diploid parents. Degrees of generational sterility and incompatibility, zygotic lethals and genetic factors which affect rate of growth are probably concerned in this variation which occurs within the diploid varieties, but as shown in Table XI and in the accompanying graphs the offspring from diploids \times diploids are invariably more uniform and vigorous than the offspring from triploids \times triploids, triploids \times diploids or diploids \times triploids.

Our colleague Dr A. A. Moffett (1931 *a*) reported upon a number of the seedlings we have raised from crosses between $2n$ and $3n$ and $3n \times 3n$ forms. The chromosomes of the seedlings Moffett examined from crosses between $2n$ and $3n$ varieties ranged in number from 37 to 47 $2n$, and those from $3n \times 3n$ from 47 to 64 $2n$. It is therefore evident that the weak and feeble growth characteristic of the majority of seedlings raised from triploid parents is associated with an aneuploid chromosome constitution.

We have previously pointed out (Crane and Lawrence, 1930) that considerable variation in the degree of generational sterility occurs both among the triploid and diploid varieties of apples, but the degree of

sterility is very much higher in the triploids than in the diploids. Pollen germination tests have been made by several investigators and their records show that the percentage of pollen germination of the triploid varieties examined ranges from 4 to 27 per cent. and that of the diploids from 46 to 98 per cent.

Since no tetraploid form of the cultivated apple is known it is probable that the triploid varieties have arisen from diploids by the functioning of an unreduced gamete. Rybin (1926) has reported triploids arising from diploid parents, and judging from its very low proportion of good pollen one of the seedlings we have raised from diploid \times diploid parents is a triploid. Nebel (1929 b) has reported that the crab apple "Kola" is a tetraploid with 68 chromosomes. Kola was raised by Hansen (1927) from crossing the wild crab apple of America with the cultivated variety Duchess of Oldenburg ($2n=34$).

DISCUSSION.

For many reasons the apple cannot be considered a favourable plant for genetic study. The length of time which elapses from seed to maturity, the space the seedlings occupy, the cost of maintenance, etc., all mitigate against any large-scale investigation unless exceptional facilities are available. With the exception of Antonowka and Rev. W. Wilks, it has not been possible to raise selfed families with any degree of success from the fifty varieties we have investigated, and most of the varieties we have used as parents have proved to be heterozygous in many respects. It is therefore obvious that larger families than we have been able to raise are desirable. In particular, families from crosses between certain varieties with similar characters, such as flat \times flat and large \times large fruits would have been of considerable value for the purpose of genetic analysis.

The results obtained are insufficient to enable definite conclusions to be formed, or to warrant a detailed discussion. Nevertheless, the results presented here for the characters studied by us are remarkably uniform in one particular. Almost without exception variation in the apple is practically continuous. The best defined characters were studied and these usually appeared to be inherited in every degree in the first generation. How can we attempt to account for this almost invariable continuous variation and wide diversity in segregation? In the first place the high basic number of seventeen chromosomes found in *Pyrus* strongly suggests that the apple is a polyploid with a consequent repetition of many genes. Secondly, the ease with which many *Pyrus* species, and even a number of

genera in the Pomoideae, may be crossed points to hybridisation being of frequent occurrence both now and in the past. This view is further supported by the fact that *Pyrus* species are difficult to classify with any degree of certainty, species merging into species with few exceptions—a condition to be expected where hybridisation is prevalent. Vavilov (1930) states that the area of the wild apple is very extensive and describes localities in Asia where they are concentrated, and where such species as *P. pumila* and *P. silvestris* are found. He reports that the wild apples found in the Caucasus are fairly small, but that the wild apples of Turkestan are characterised by their comparatively large size. Individual trees bear fruit which in quality is not inferior to that of cultivated forms, and some are of astonishingly large size and exceptional productivity. Vavilov also states that in some localities the whole scale of transition from the typical small, sour apple to the cultivated, perfectly edible forms may be observed.

In the third place, as we have previously shown (Crane and Lawrence, 1929) a high degree of self-sterility is of common occurrence in the cultivated apple, not only as measured by the percentage of fruit set but as seen in the low viability of the seeds and lack of vigour in the seedlings raised from self-pollinations, conditions which almost certainly may be traced ultimately to self-sterility. It is therefore apparent that in the cultivated apple self-sterility must be a major factor in regard to hybridisation, since out-crossing is enforced to a considerable extent and a high degree of heterozygosity maintained. Thus polyploidy, hybridisation and self-sterility all combine to produce a complex genetic constitution in the apple.

The larger implications of this statement cannot be realised unless the chromosome constitution and probable origin of *Pyrus* are considered in greater detail—indeed they are vital factors to a proper understanding of the genetical results.

With the exception of the Pomoideae, the basic chromosome numbers of the genera of Rosaceae are 7, 8 and 9, and of these 7 is by far the commonest. Three different theories have been advanced to account for the anomalous chromosome number of the Pomoideae. According to Nebel (1929*a*) the genus *Pyrus* (*Malus*) is a halved pentaploid with 7 as the basic chromosome number. Darlington and Moffett (1930) and Moffett (1931*a*) have concluded from their observations that *Pyrus* is derived from an original basic number of 7, but for reasons quite different from those of Nebel. Sax (1931–2) believes that *Pyrus* is an allotetraploid which may have arisen from hybridisation of species with

8 and 9 pairs of chromosomes respectively, coupled with doubling of the chromosome number to give $2n=34$.

As we show later, the origin of the Pomoideae from a basic number of 7 is implicit in Sax's theory as well as explicit in the other two, but since Sax has elaborated his views we will first examine the evidence for the origin from $n=8$ and $n=9$ forms. If the apple originated from the crossing of diploid forms with 8 and 9 pairs of chromosomes, the fact that multivalent association of the chromosomes is not at all common in the apple indicates either (1) that the original parental species were already considerably differentiated in genetic constitution, or (2) that a sufficient period of time has elapsed since the origin of *Pyrus* for chromosome differentiation to proceed to the point where multivalent pairing is no longer of general occurrence, *i.e.* a high degree of genic differentiation has occurred. On either view the inference is that in an allotetraploid which had arisen from simple diploid forms inheritance would be likely to exhibit Mendelian rather than tetrasomic segregation, since the genes would not be in identical fours, but in well-differentiated pairs as in diploid individuals. As we have shown, the majority of the characters we have studied grade imperceptibly, not only in their expression but in inheritance—conditions which point to a highly complex genetic constitution.

The cytological investigations of Darlington and Moffett seem to provide evidence as to the precise nature of this complex constitution of the apple. Briefly, the 34 chromosomes usually associate in pairs at meiosis, but these bivalents are found to form a varying number of secondary groups of 2 or 3 bivalents each. The maximum secondary association observed was 3 groups of 3 bivalents and 4 groups of 2, most cells showing one or two groups of 2 or 3 bivalents each. Now secondary association has been shown, by Lawrence (1931 *a, b*), Müntzing (1933) and Meurman (1933) to indicate chromosome homologies more remote than that evident in normal pairing, and Darlington and Moffett have interpreted secondary association observed in the apple as indicating triplication of 3 chromosome pairs and duplication of 4 pairs of an original number of 7 pairs, so that the group of species are complex allopolyploids described as secondary polyploids.

Whatever the precise evolution of *Pyrus* may have been, the clearness and consistency of the secondary association seem to permit of no other explanation than that some of the chromosome pairs have been triplicated. Therefore if the apple arose from the crossing of two species having $n=8$ and $n=9$ chromosomes, then these species themselves must have had certain of the chromosomes repeated, *i.e.* they were trisomic or

tetrasomic in respect of a number of genes. The validity of this hypothesis is further supported by the interesting observations of Sax (1932) that the genera *Rhodotypus* and *Neriusia* each with 9 pairs of chromosomes have 2 pairs of chromosomes "so closely associated that counts are difficult." This suggests that such hyperdiploids could and do exist.

Whatever the exact details of the process therefore, whether from hybridisation of types with seven as the basic number or from later types with 8 and 9 chromosomes, the available evidence points to an ancestral and original basic number of 7 chromosomes, which still show their affinities, and therefore homologies, in the secondary association found at meiosis.

Since out of a total of 34 chromosomes in the apple there are 18 chromosomes (6×3), any one of which is more or less similar to five others, the chances are slightly more than even that a gene will be hexasomic or have 1-5 other genes similar but slightly different from itself. For example, if one of the chromosome types which is represented 6 times be designated by the letter **A**, and this chromosome carries a gene for fruit shape or colour, then each of the other five **A** chromosomes might carry (1) identical genes acting in a cumulative manner, thus giving 7 different genotypes along with the recessive allelomorphs, or (2) some identical and some slightly different genes, all of them governing shape or colour but in slightly different ways, thus giving rise to a far greater complexity than is found in an ordinary allotetraploid. Such differentiation of genes within a given chromosome set is known to occur. For example, in the octoploid garden dahlia (Lawrence, 1931 c; Lawrence and Scott-Moncrieff, in the press) each chromosome type is represented 8 times, but of these 4 chromosomes have been derived by one line of descent and 4 by another. In the one half-set of 4 chromosomes a gene necessary for anthocyanin production is cumulative in expression and incompletely dominant; in the other half set of homologous chromosomes the anthocyanin gene is non-cumulative in expression, completely dominant and more pronounced in its effect. The combined effects of these half-sets whose inheritance is tetrasomic lead to a very complex range of colours.

Generally speaking the older a polyploid the more simple and well defined will be the inheritance and expression of its characters. By this token the apple must have originated comparatively recently—a deduction which is in accordance with the comparative lack of differentiation of the morphological characters of the Pomoideae, the ease of inter-specific hybridisation and the frequency of fertile species hybrids.

Small as many of the families raised in these experiments have been, they should have given some indication of the mode of inheritance even if tetrasomic segregation had occurred. In this connection it is noteworthy that in our experience, Crane (1921), the characters and their inheritance in the hexaploid domestic plum are far less continuous than in the apple. With the exception of albinism, the complete lack of discontinuous variation encountered in these studies points to an unusually complex genetical constitution, which can be accounted for on the hypothesis that certain pairs of chromosomes of the apple have been triplicated.

The imperceptible gradations in F_1 progenies may result from the action of a number of identical genes whose expression in the phenotype is more or less cumulative, or to the action of similar (but not identical) genes whose expression is differential and cumulative. When a number of cumulative and differential factors govern the same character, the expression of dominance is essentially more variable. Indeed, certain balances may suggest that a character is recessive whereas others may point to dominance. Difficulty in deciding dominance has been encountered in these studies, and it is not improbable that it arises from some such cause.

Among varieties of apples varying degrees of self-incompatibility occur; we have investigated fifty varieties, and only two have entirely failed upon selfing and two combinations upon crossing. This contrasts sharply with the results we have obtained from investigating a similar number of cherries and plums. In the diploid cherries ($2n=16$) 100 per cent. of the varieties tested were completely self-incompatible and 70 per cent. were reciprocally cross-incompatible. Within the groups all combinations are incompatible, no one-way incompatibility occurring. In the hexaploid plums ($2n=48$) 40 per cent. of the varieties were completely self-incompatible and 13.5 per cent. cross-incompatible. Compared with the diploid cherries, not only are the results with plums much more complex, but fewer groups occur, and within them incompatibility is not complete as in the case of the cherries. The groups include self-incompatible, partially cross- and self-compatible, cross-incompatible and cross-compatible combinations, and reciprocal differences involving both partial and complete incompatibility.

In apples incompatibility grades imperceptibly, and the absence of incompatible groups is noteworthy. The only two examples of cross-incompatibility we have found differ reciprocally, being compatible in one direction and incompatible in the other. We have previously shown

(Crane and Lawrence, 1929; Lawrence, 1930) that such one-way incompatibility is a phenomenon most frequently associated with polyploidy, and an analysis of our incompatibility results suggest that the nuclear constitution of the apple with 34 chromosomes is more complex than that of the hexaploid plums with 48.

SUMMARY.

The following characters of the apple have been investigated, and an attempt made to analyse their mode of inheritance:

- (1) Ground colour.
- (2) Anthocyanin colour.
- (3) Flesh colour.
- (4) Fruit surface.
- (5) Fruit size.
- (6) Fruit shape.
- (7) Flavour.
- (8) Time of ripening.
- (9) Albinism.
- (10) Root-burr formation.

With the exception of albinism, no sharply discontinuous segregation has occurred. It is suggested that the wide and intergrading variation which occurs with respect to most of the characters studied is due to the action of polymeric factors. The probable origin and constitution of the apple are discussed in relation to the genetic data.

The results obtained from crosses between diploid and triploid and between triploid and triploid varieties are described. It is shown that although an occasional vigorous seedling may arise from such crosses, the large majority make very poor growth, and this lack of vigour is associated with an aneuploid chromosome constitution.

ACKNOWLEDGMENTS.

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EXPLANATION OF PLATES IX AND X.

PLATE IX.

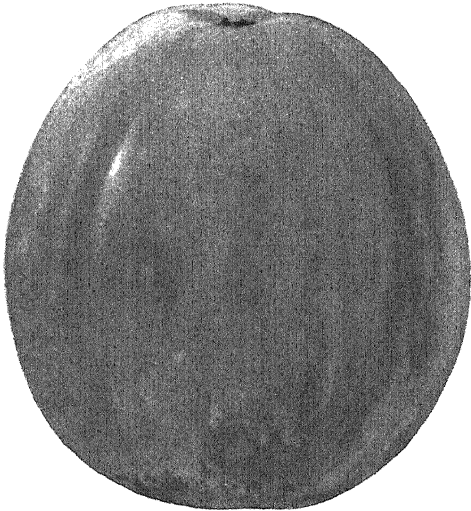
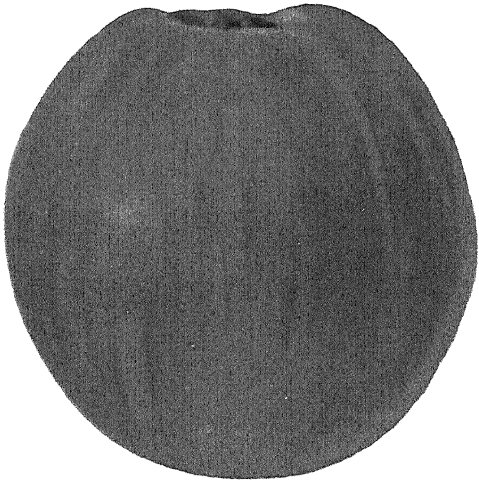
Top. Duchess Favourite × Cox's Orange Pippin. Seedling No. 209, red on yellow ground.

Bottom. Antonowka × Worcester Pearmain. Seedling No. 413, red on cream ground. From drawings by Mr C. H. Osterstock.

The colour of seedling 209 is approximately correct, but in seedling 413 there is a little too much yellow.

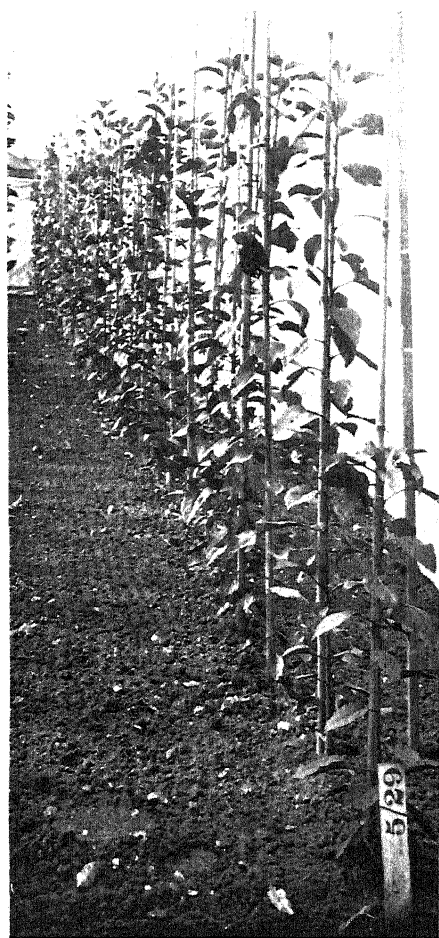
PLATE X.

Apple seedlings six months from germination. A. triploid × diploid and triploid × triploid. B. diploid × diploid.





A



B

STUDIES ON THE GENETICALLY INERT REGION OF THE X-CHROMOSOME OF *DROSOPHILA*.

I. BEHAVIOUR OF AN X-CHROMOSOME DEFICIENT FOR A PART OF ITS INERT REGION.

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(With One Text-figure.)

I. INTRODUCTION.

THE recent works of Muller and Painter (see Painter, 1931; Painter and Muller, 1932; Muller and Painter, 1932) have shown that a considerable part (probably about a half) of the X-chromosome of *D. melanogaster* is genetically inert. Further, it has been made clear by the same works that this inert part of the X-chromosome is homologous with the Y-chromosome. These discoveries, which have been confirmed by Dobzhansky (1932), raise a number of important problems concerning the structure and evolution of the sex-chromosomes of *Drosophila*. An experimental study of some of these problems was undertaken by the writer. The present work forms the first part of this investigation.

II. MATERIAL¹.

As material for the present work an X-chromosome of *D. melanogaster* was used, obtained through crossing-over of two differently inverted X-chromosomes, the chromosome $y-sc^4$ (found by I. I. Agol (1929)) and the chromosome sc^8-w^a (found by B. N. Sidorov (1931)).

In the chromosome $y-sc^4$ the left break of the inversion passes between the genes sc and pn and the right one lies to the right of f but to the left of bb . The $y-sc^4$ chromosome used in the present work besides y and sc carried the genes v and f introduced there by double crossing-over.

¹ For the convenience of the reader the names of the characters referred to in this paper are given below. The figures in brackets after each character indicate the position of its locus on Chr. I.

ach = achaete (0.0); B = bar (57.0); b = bobbed (70.0); Cl = suppressor of $cr.$ and lethal; f = forked (56.5); pn = prune (0.8); sc = scute (0.0); v = vermilion (33.0); w^a = apricot (1.5); w^e = eosin (1.5); y = yellow (0.0).

In the chromosome sc^8-w^a the left break of the inversion passes likewise just to the right of sc . The right break, as has been shown by M. M. Kamshilov (unpublished), lies to the right of bb , between this locus and the point of attachment of the spindle fibre.

In a cross of a $\frac{y\ sc^4\ f\ v}{sc^8\ w^a}$ female with $y-w^a$ males besides the non-cross-over classes of males there were obtained some males showing the characters y , sc , w^a , v and f . These males evidently were the result of a single crossing-over between the loci of v and w^a of the $y-sc^4$ and sc^8-w^a chromosomes. A chromosome arisen through a single crossing-over of two differently inverted chromosomes must carry a duplication and a deficiency. In the given case the duplication should have included a small piece of the chromosome immediately to the right of sc (if only the left breaks of both inversions do not coincide exactly). The deficiency should have included the region between the right breaks of both the inversions and consequently the locus of bb .

The fact that males carrying this cross-over X-chromosome proved to be viable is best explained on the assumption that the deficiency in it includes only such parts as are homologous with the Y-chromosome, and that therefore the presence of the Y-chromosome inhibits the lethal action of the deficiency. To test this supposition males carrying the X-chromosome in question were crossed with females homozygous for bobbed and with females homozygous for carnation. The F_1 females from crosses with bb showed a sharp exaggeration of this character, thus supporting the above supposition. Crosses with carnation females showed that the deficiency does not include this locus. Further studies have shown that the X-chromosome with the deficiency is lethal in homozygous females and in females heterozygous for the lethal allelomorph of bb (bb^1), but exerts no lethal effect in case such females carry a Y-chromosome. All this leads to the conclusion that the X-chromosome studied has a deficiency including bb and no other known loci. Henceforth this chromosome, carrying, as has been already stated, the genes y , sc^4 , w^a , v and f , will be referred to briefly as the " bb -def" chromosome.

III. THE GENETIC BEHAVIOUR OF THE CHROMOSOME " bb -def" IN XY MALES.

Assuming that the X-chromosome investigated has a deficiency for the bb locus, the question arose whether this deficiency is limited to this locus or includes a certain part of the inert region of the X-chromosome

located between the gene bb and the point of attachment of the spindle fibre.

If the " bb -def" chromosome carried a deficiency, including not only bb but also a part of its inert region, its synaptic affinity to the Y -chromosome would be more or less weakened. This weakening of the synaptic affinity could be most easily detected in males, where it would lead to an increase of the frequency of primary non-disjunction of the X and Y -chromosomes.

In order to decide this question, males carrying the " bb -def" X -chromosome were crossed with $\frac{bb}{bb^1}$ females. This structure of females was selected because in the stock at our disposal the character bb often overlapped the wild type, even in XX females. Hence some of the P females might carry a Y -chromosome, thus marring the results of the series. On the other hand, the females used in the experiment always express bb very sharply owing to the exaggeration of this character conditioned by the presence of bb^1 , and contamination by XXY females can therefore be easily avoided.

In case of primary non-disjunction of the X and Y -chromosomes a male should, besides regular X and Y gametes, give also exceptional gametes carrying both the X and Y -chromosomes, or neither of them. Spermatozoa with the X -chromosome fertilising eggs carrying bb should give bb females with an exaggerated manifestation of the character. The same spermatozoa fertilising eggs carrying bb^1 should give non-viable zygotes. Spermatozoa with the Y -chromosome fertilising eggs of both types should give wild (non- bb) males. And finally spermatozoa deprived of both sex-chromosomes on meeting with eggs carrying bb should give bb males, and with eggs carrying bb^1 non-viable zygotes (Table I). Also

TABLE I.

Eggs	Sperms			
	Regular		Exceptional	
	-" bb -def"	→	" bb -def"	O
Regular				
bb	♀ bb	♂ +	♀ +	♂ bb
bb^1	Dies	♂ +	♀ +	Dies
Exceptional				
bb }	Dies	♀ +	Dies	♀ bb
bb^1 }				
O	Dies	Dies	♂ " bb -def"	Dies

in a small number of cases there might occur primary non-disjunction of

the *X*-chromosomes in the mother, which would result in exceptional wild (non-*bb*) females, not distinguishable from females obtained through fertilisation of normal eggs by spermatozoa having both sex-chromosomes (*XY*). There might also result, though but very rarely, *bb* females through fertilisation of exceptional *XX* eggs by exceptional spermatozoa of the *O* type, as well as patroclinal males, obtained through fertilisation of exceptional *XX* eggs by exceptional *XY* spermatozoa.

Crosses of this type gave the results summarised in Table II. They

TABLE II.

Phenotype	♀ <i>bb</i>	♀ +	♂ +	♂ <i>bb</i>	Total
Number of flies	294	272	2292	430	3288

show that males carrying the "*bb-def*" chromosome actually give a considerable number of exceptional gametes having both *X* and *Y*-chromosomes, or neither of them. In other words, these results indicate a considerable weakening of the synaptic affinity between the "*bb-def*" *X* and the *Y*-chromosomes. Hence it is probable that this *X*-chromosome carries a deficiency including not only bobbed, but also a part of the inert region homologous with the *Y*-chromosome.

The data summarised in Table II offer the possibility of determining among the gametes produced by a "*bb-def*" male the ratio of the spermatozoa carrying the *Y*-chromosome to the exceptional spermatozoa lacking both the sex-chromosomes. The number of spermatozoa carrying the *Y*-chromosome can be estimated directly from the number of wild males appearing in the offspring. The number of exceptional *O* spermatozoa can be obtained by doubling the number of *bb* males. In this way we arrive at a ratio of 37.52 spermatozoa lacking the *X* and *Y*-chromosomes to 100 carrying the *Y*-chromosome. Any error in this ratio would be probably to the advantage of the spermatozoa with the *Y*-chromosome, since there may be some decline of viability in the *bb* males. Thus the number of exceptional spermatozoa without the *X* and *Y*-chromosomes is probably not exaggerated and may even be diminished.

Further, the results of crosses of this series may be used for determining the number of exceptional spermatozoa which carry both the *X* and *Y*-chromosomes. The calculation of the ratio of such spermatozoa to the spermatozoa carrying only the *X*-chromosome on the basis of the ratio of wild females to *bb* females is here impossible owing to $\frac{bb}{bb-def}$ females possessing a greatly decreased viability (as shown also by separate crosses). But we can calculate the ratio of the spermatozoa carrying both

the X and Y -chromosomes to those carrying only the Y -chromosome. From the number of wild males and wild females among the offspring, we obtain a ratio of 12.83 spermatozoa of the XY type to 100 carrying only the Y -chromosome. It is necessary to remember that the number of exceptional spermatozoa of the XY type arrived at in this way may be somewhat exaggerated; firstly, because the males of *Drosophila* generally have a somewhat lesser viability than the females and, secondly, because of the possibility that some of the wild females in the crosses under consideration could have been produced through fertilisation of XX eggs received as a result of primary non-disjunction in their mother, by Y spermatozoa. But any error due to this latter reason would be insignificant owing to the scarcity of primary non-disjunction even in bb females. In fact, out of 89 wild F_1 females tested, not one was produced as a result of primary non-disjunction in its mother.

The determination of the percentage of spermatozoa carrying only the X -chromosome in the series of crosses does not appear possible except on the assumption that the number of X spermatozoa is equal to that of Y spermatozoa.

The considerable preponderance among the gametes of " bb -def" males of exceptional spermatozoa of the O type over exceptional spermatozoa of the XY type may be explained upon the supposition that unsynapsed X and Y -chromosomes are often incapable of reaching either of the daughter nuclei, and are consequently easily lost, just as it is the case, according to the generally accepted assumption, with the unsynapsed X 's during primary non-disjunction in the females of *Drosophila* (Bridges, 1916; Morgan, Bridges and Sturtevant, 1925).

Besides the crosses recorded in Table II there were four others which gave exclusively wild males and females. These crosses will be considered later on.

In order to verify the conclusions arrived at as a result of this series of crosses two more sets of experiments were undertaken.

One of these was devoted to verifying the ratio between the number of spermatozoa carrying the Y -chromosome and the number of spermatozoa lacking both the X and Y -chromosomes. This series consisted of crosses of " bb -def" males with females from the attached- X stock homozygous for sc and w .

In the offspring from such crosses two types of females would be obtained: \widehat{XXY} females, resulting from Y spermatozoa, and \widehat{XX} females, resulting from exceptional spermatozoa without the X and Y -chromosomes (Table III).

TABLE III.

Eggs	Sperms			
	Regular		Exceptional	
	X	Y	XY	0
\widehat{XX} Y	Dies ♂ XY	♀ $\widehat{XX}Y$ Dies	Dies ♂ XY Y	♀ \widehat{XX} Dies

In order to determine the ratio of these two types of females the F_1 females from such crosses were mated with $w^e bb^1$ males. The \widehat{XX} females could be thus separated from the $\widehat{XX}Y$ females, the former giving in their offspring no males owing to the lethal action of bb^1 in the XO male, and the latter producing offspring of both sexes.

A study of 317 F_1 females derived from ten " bb -def" males showed that 229 of them were produced as a result of fertilisation of the egg by a spermatozoon carrying the Y-chromosome, and 88 as a result of fertilisation of the egg by an exceptional spermatozoon of the O type (Table IV).

TABLE IV.

Structure of F_1 females	♀ $\widehat{XX}Y$	♀ \widehat{XX}	Total
Number of females	229	88	317

The ratio between the number of exceptional spermatozoa of this type and the number of Y spermatozoa produced by " bb -def" males is according to this series of crosses equal to 38.43 : 100—a ratio close to that obtained in the first series of experiments.

The second control series was to determine the ratio between the number of XY and the number of X spermatozoa produced by " bb -def" males. In order to obtain this ratio " bb -def" males were crossed with $\frac{ClB}{w^e bb^1}$ females.

In these crosses, spermatozoa carrying only the X-chromosome should give equal numbers of sc -v-B females and non-viable zygotes: spermatozoa carrying both the X and Y-chromosomes should give equal numbers of sc -v-B and w^e females: spermatozoa with the Y-chromosome should give equal numbers of w^e males and non-viable zygotes: while spermatozoa lacking both the X and Y-chromosomes should produce only non-viable zygotes (Table V). Besides, in case of primary non-disjunction in the females, which is very frequent in the presence of the ClB chromosome, there should have appeared in the offspring exceptional bar females (non- sc , non-v), and in a very small number there should

TABLE V.

	Sperms			
	Regular		Exceptional	
Eggs	"bb-def"	→	"bb-def"	O
Regular				
CLB	♀ <i>sc-v-B</i>	Dies	♀ <i>sc-v-B</i>	Dies
<i>w^e bbⁱ</i>	Dies	♂ <i>w^e</i>	♀ <i>w^a</i>	Dies
Exceptional				
CLB	Dies	♀ <i>B</i>	Dies	♀ <i>B</i>
<i>w^e bbⁱ</i>				
O	Dies	Dies	♂ "bb-def"	Dies

appear patroclinous "bb-def" males, the latter as a result of fertilisation of exceptional eggs without the X's by exceptional XY spermatozoa.

TABLE VI.

Phenotype	♀ <i>sc-v-B</i>	♀ <i>w^a</i>	♀ <i>B</i>	♂ <i>w^e</i>	♂ "bb-def"	Total
Number of flies	5886	546	879	4240	72	11623

The results of this series of experiments are presented in Table VI from which it is possible to determine the number of spermatozoa of the XY type by means of doubling the number of *w^a* females, and the number of spermatozoa of the X type by subtracting from the number of *sc-v-B* females the number of *w^a* females. This gives a ratio of 10.22 spermatozoa carrying both the X and Y-chromosomes to 100 carrying the X-chromosome.

This ratio of XY : X spermatozoa is somewhat lower than the ratio of XY : Y spermatozoa determined from the first series of experiments, probably indicating a decreased viability of the males in the first series. However, the two ratios are so close that they suggest numerical equality between the spermatozoa of the X and of the Y types, and this assumption is strengthened by other considerations. In the $\frac{CLB}{w^e bb^i}$ series were obtained 5340 females resulting from eggs fertilised by X spermatozoa and 4240 males resulting from eggs fertilised by Y spermatozoa. The fact that the number of males is less than expected can be explained either by their decreased viability (as compared with that of the females) or by supposing that the "bb-def" males give fewer spermatozoa of the Y type than of the X type (which could occur if, for instance, the Y-chromosome is more apt to be lost during meiosis than the X-chromosome).

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In order to determine which of these two assumptions is correct, $\frac{ClB}{w^e bb^i}$ females were crossed with $y-ach-v$ males.

The results of this series (where a ratio of 2 ♀ : 1 ♂ was to be expected) show that the w^e males possess a somewhat lower viability than the $v-B$ females (Table VII). As compared with the viability of $v-B$ females, that

TABLE VII.

Phenotype	♀ $v-B$	♀ +	♀ B	♂ w^e	♂ $y-ach-v$	Total
Number of flies	564	574	71	463	48	1720

of w^e males is here equal to 80.7 per cent. Calculating now, with this correction for their lower viability, the number of w^e males expected in the offspring of $\frac{ClB}{w^e bb^i}$ females crossed with “ $bb-def$ ” males, upon the assumption of numerical equality of the X and Y types of spermatozoa formed by “ $bb-def$ ” males, we arrive at the figure 4309. This number differs from that actually obtained only within the limits of statistical error, *i.e.* the difference between them amounts only to 0.99 of its standard error. This confirms the supposition that the “ $bb-def$ ” males produce X and Y spermatozoa in equal quantities.

In Table VI are not included six crosses that showed secondary non-disjunction of the X 's in their mother. These crosses are easily distinguishable from crosses resulting from an XX female, because in cases of secondary non-disjunction they show an extraordinarily high percentage of exceptions (which is a general characteristic of all XXY females heterozygous for the ClB chromosome). In these crosses there were obtained about 30 per cent. of exceptions among the females and about 50 per cent. among the males, while for primary non-disjunction in similar crosses the percentage of exceptions was equal to 14.9 in the females and only 1.7 in the males. The typicalness of the offspring from XXY females

TABLE VIII.

No.	♀ $sc-v-B$	♀ w^a	♀ B	♂ w^e	♂ “ $bb-def$ ”	Total
4911	41	19	42	32	24	158
4919	57	25	44	37	36	199
5043	29	14	38	30	18	129
5050	50	21	51	40	32	194
5076	41	18	37	46	22	164
5084	35	16	34	31	19	135
Total	253	113	246	216	151	979

is seen in Table VIII (compare with Table VI). The fact that in both tables are included only crosses which yielded more than a hundred flies,

and the sharp difference observed between the percentages of primary and secondary exceptions, is a guarantee against the error that might have crept in it, if crosses yielded by *XXY* females had been included in Table VI.

Besides the six crosses which showed secondary non-disjunction, five more crosses of the same series giving an exceptionally high percentage of *w^a* females are not included in Table VI. Each of these crosses gave approximately equal numbers of *sc-v-B* and *w^a* females (Table IX), a

TABLE IX.

No.	♀ <i>sc-v-B</i>	♀ <i>w^a</i>	♀ <i>B</i>	♂ <i>w^a</i>	♂ " <i>bb-def</i> "	Total
5045	26	18	5	28	2	79
5047	31	36	5	34	1	107
5061	22	24	8	29	2	85
5066	28	23	1	27	—	79
5089	30	27	3	26	3	89
Total	137	128	22	144	8	439

result markedly different from the results observed in the rest of the crosses of this series. None of these five crosses showed the high percentage of exceptions characteristic for secondary non-disjunction, and all five were no doubt derived from *XX* females. These crosses will be considered later on.

The results of all these series show that the synaptic affinity between the *X*-chromosome studied and the *Y*-chromosome is considerably weakened. In order to ascertain whether this weakening of the synaptic affinity is due to the presence in the "*bb-def*" *X*-chromosome of a deficiency for a part of its inert region it is necessary to prove that this weakening was not characteristic of the initial chromosomes *y-sc⁴* and *sc⁸-w^a*, i.e. that it arose simultaneously with the appearance of the deficiency. To clear up this question *y-sc⁴* and *sc⁸-w^a* males were crossed with $\frac{bb}{bb^1}$ females. The results of these crosses (Table X for *y-sc⁴* males and Table XI for *sc⁸-w^a* males) show that the synaptic affinity of these

TABLE X.

Phenotype	♀ +	♂ +	Total
Number of flies	1122	963	2085

TABLE XI.

Phenotype	♀ +	♂ +	♂ <i>sc⁸w^a</i>	Total
Number of flies	1384	1136	2	2522

chromosomes and the *Y*-chromosome is entirely normal. In neither series, in each of which about a thousand *F₁* males were obtained were

any *XO* males to be found. Thus it can be considered as ascertained that the weakening of the synaptic affinity between the "*bb-def*" *X*-chromosome and the *Y*-chromosome is actually to be explained by the deficiency present in the former.

It is interesting that the only two cases described before of high primary non-disjunction of the *X* and *Y*-chromosomes in the males of *Drosophila* were also observed in lines, known to carry a deficiency for a part of the inert region (Sivertzev-Dobzhansky and Dobzhansky, 1933), or probably carrying such a deficiency (Kuhn, 1929; Stern, 1930). Unfortunately, these cases are described in insufficient detail to form a fuller conception of the course of synapsis and the reduction division.

IV. SYNAPSIS IN *XY* "*bb-def*" MALES.

From the above data it is possible to form a conception of the course of synapsis and disjunction of the *X* and *Y*-chromosomes in the "*bb-def*" males.

Summarising the data of all the series we get the following picture (Table XII):

TABLE XII.

Series	Sperms			
	Regular		Exceptional	
	<i>X</i>	<i>Y</i>	<i>XY</i>	<i>O</i>
♀ $\frac{bb}{bb^1}$	—	2292	—	860
♀ $\frac{XXY}{bb^1}$	—	229	—	88
♀ $\frac{ClB}{w^e bb^1}$	5340	—	546	—

From the series ♀ $\frac{bb}{bb^1} \times \text{♂ "bb-def"}$ and the series with the attached-*X* females we see that the ratio of *Y* to *O* spermatozoa given by "*bb-def*" males is equal to 100 : 37.60. From the series ♀ $\frac{ClB}{w^e bb^1} \times \text{♂ "bb-def"}$ we find that the ratio of *X* to *XY* spermatozoa produced by the same males is equal to 100 : 10.22. Also, the same series shows that the "*bb-def*" males form equal numbers of *X* and *Y* spermatozoa (see discussion on p. 310). Expressing the number of each type of spermatozoa in percentages of the total number of spermatozoa given by "*bb-def*" males we obtain the following picture (Table XIII): 40.35 *X*; 40.35 *Y*; 4.13 *XY*; 15.17 *O*.

Let us assume now that during the spermatogenesis in "*bb-def*" males the *X* and *Y*-chromosomes synapse (moving thereupon to different

TABLE XIII.

Types of spermatozoa	Regular		Exceptional	
	<i>X</i>	<i>Y</i>	<i>XY</i>	<i>O</i>
Percent to total number of spermatozoa	40.35	40.35	4.13	15.17

poles) in p per cent. of cases. Then the number of cases when the X and Y are left unsynapsed will be equal to $(100-p)$ per cent. If the X and Y -chromosomes have not conjugated they will be distributed at random, *i.e.* with an equal probability of forming X , Y , XY and O gametes, each type in $\frac{100-p}{4}$ per cent.

Let us assume further that in the absence of conjugation of the X and Y -chromosomes, each of the unsynapsed chromosomes can be lost with a probability q .¹ Then $q \frac{100-p}{4}$ per cent. gametes of type X and as many gametes of type Y will lose their sex-chromosomes and will move into type O , thus increasing it by $2q \frac{100-p}{4}$ per cent. Further, $q \frac{100-p}{4}$ per cent. gametes of type XY will lose their Y and will move into type X , and as many gametes of the same type will lose their X and will move into type Y . Finally, $q^2 \frac{100-p}{4}$ per cent. gametes of the type XY will lose both their sex-chromosomes and will move into type O . Thus the number of spermatozoa of each type will be determined according to the following formulae:

$$\text{Type } X = \left(\frac{p}{2} + \frac{100-p}{4} \right) \text{ per cent.} \quad \dots(1)$$

$$\text{Type } Y = \left(\frac{p}{2} + \frac{100-p}{4} \right) \text{ per cent.} \quad \dots(2)$$

$$\text{Type } XY = \left(\frac{100-p}{4} - 2q \frac{100-p}{4} - q^2 \frac{100-p}{4} \right) \text{ per cent.} \quad \dots(3)$$

$$\text{Type } O = \left(\frac{100-p}{4} + 2q \frac{100-p}{4} + q^2 \frac{100-p}{4} \right) \text{ per cent.} \quad \dots(4)$$

The value of p (the percentage of synapsis between X and Y) may be determined from (1) and (2). Substituting in these formulae the values of types X and Y shown in Table XIII we find it equal to 61.40 per cent. The value of q (the probability of the loss of an unsynapsed X or Y) may

¹ The fact that "bb-def" males form equal numbers of X and Y spermatozoa (see Table VI and the discussion of it) makes it evident that the unsynapsed X and Y -chromosomes are lost with equal frequency.

be determined both from (3) and (4). Determining the value of q by substituting in (3) the value of type XY shown in Table XIII, we find it equal to 0.254. Determining it by substituting into formula (4) the value of type O shown in Table XIII, we find it likewise equal to 0.254. Such an exact coincidence of the values of q calculated from both (3) and (4) is a good confirmation of the correctness of the calculations, and shows that the percentages of the different types of spermatozoa arrived at in the experiments and grouped together in Table XIII very nearly reflect the actual picture.

V. THE GENETIC BEHAVIOUR OF THE CHROMOSOME " bb -def" IN XY MALES.

Bridges (1916) and Stern (1927, 1929, 1930) have shown that during meiosis in the XY males of *D. melanogaster* all three chromosomes are distributed at random, leading thus to the formation of different types of gametes in the ratio $1X : 1XY : 2XY : 2Y$.

The fact that the chromosome " bb -def" has a deficiency for a part of its inert region homologous with the Y -chromosome, and that owing to this its synaptic affinity to the Y -chromosome is considerably weakened, makes it likely that in XY males carrying this chromosome the X and Y -chromosomes will not be any more distributed at random. It must be supposed that in such males synapsis will most frequently include both the Y -chromosomes, whose synaptic affinity is normal, and that in such cases the X -chromosome will be distributed at random. Heterosynapsis of the X and Y -chromosomes is to be expected to occur much more rarely, because of the weakening of the synaptic affinity to the Y -chromosome characteristic of the " bb -def" X . In other words, the reduction in such XY males will take place on the same principle as in normal XXY females, the main difference being that the Y -chromosomes will take the place of the X -chromosomes, and the X -chromosome will take the place of the Y -chromosome.

The high frequency of primary non-disjunction in " bb -def" XY males, resulting in the formation of a considerable number of XY spermatozoa, should lead to the appearance of a considerable number of XY " bb -def" males in the stock (which was maintained through crosses with attached- X females). In point of fact, in the series ♀ $\frac{bb}{bb^1} \times \text{♂ "bb-def"}$ there appeared four crosses (see p. 306), and in the series ♀ $\frac{CLB}{w^e bb^1} \times \text{♂ "bb-def"}$ five crosses (see Table IX), the results of which are explicable on the assump-

tion that they were produced by $XY Y$ males. The fact that in the first series all these males gave only wild (non- bb) flies, and in the second series almost equal numbers of w^a and $sc-v-B$ females, leads to the supposition that such males give spermatozoa chiefly of the types XY and Y , and consequently that there is no random assortment of the sex-chromosomes during the reduction division.

To check this supposition, females obtained in these crosses were tested by crossing them to $w^e bb^1$ males. Altogether 21 wild females were tested from the first series and 36 $sc-v-B$ females from the second. If during meiosis in $XY Y$ " bb -def" males the Y -chromosomes conjugated always with one another it might be expected that all these females would possess a Y -chromosome and show secondary non-disjunction. If, on the contrary, the distribution of the X and Y -chromosomes in " bb -def" $XY Y$ males occurred at random, one-third of these females would have the structure XX . Out of 57 females investigated only one $sc-v-B$ female showed no secondary exceptions in her offspring. This fact speaks for the correctness of the hypothesis of the non-random distribution of the X and Y -chromosomes at the reduction division of $XY Y$ " bb -def" males. Because of the weakening of the synapctic affinity between the X and Y -chromosomes in the presence of normal synapctic affinity between the Y 's, these males give almost exclusively spermatozoa of two types only, viz. XY and Y .

It is necessary to note that in the crosses of $XY Y$ " bb -def" males with $\frac{ClB}{w^e bb^1}$ females the percentage of w^e males in the offspring (Table IX) is considerably higher than in crosses of similar females with XY " bb -def" males (Table VI). This probably indicates that during spermatogenesis in $XY Y$ " bb -def" males the unsynapsed X is often lost, leading to an increase of the relative number of Y spermatozoa formed.

VI. CYTOLOGICAL DEMONSTRATION OF DEFICIENCY IN THE " bb -def" X -CHROMOSOME.

The considerable cytological length of the inert region of the X -chromosome of *D. melanogaster* and the great weakening of the synapctic affinity between the " bb -def" X -chromosome and the Y -chromosomes led us to suppose that the deficiency present in the former might be observed cytologically. In order to compare the cytological length of the " bb -def" X -chromosome with that of the normal X -chromosome, a cytological study of these chromosomes was undertaken. The ovaries of " $\frac{sc-w}{bb$ -def"

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pupae ready to hatch were fixed after Painter, and the preparations stained with iron haematoxylin. Examination of the X-chromosomes of several good metaphase plates (four of which are represented in Fig. 1) showed that in all cases one of the X-chromosomes was noticeably shorter than the other. The measurements of these chromosomes leads to the conclusion that the "bb-def" X is approximately one-fourth shorter than the normal X-chromosome.

Thus, the cytological analysis of the "bb-def" chromosome confirms the presence of the deficiency discovered by genetic methods.

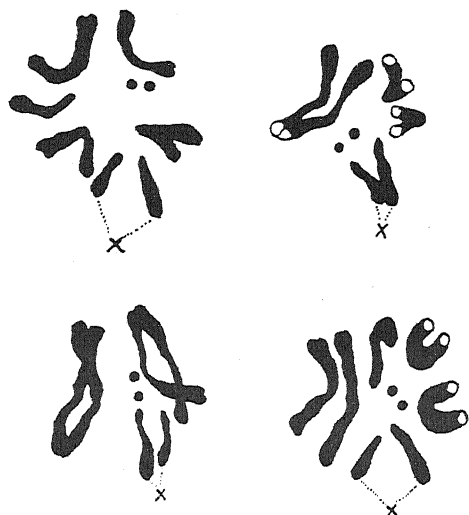


Fig. 1. All drawings represent oögonial metaphase plates and are done at the level of the work-table, with the aid of the camera lucida. The magnification is Zeiss objective 120 (1.5), and comp. oc. 30.

VII. DISCUSSION.

The above study of a deficiency in the genetically inert part of the X-chromosome confirms the views expressed by Muller and Painter concerning the homology of the inert part of the X-chromosome with the Y-chromosome. Moreover, it permits the verification of a highly important suggestion advanced by Muller and Painter (1932), according to which this inert region of the X-chromosome, as well as the Y-chromosome of *D. melanogaster*, contain in their present state few or no genes capable of mutating (with the exception of *bb*). If any genes capable of undergoing mutation were present in the part of Y-chromosome homologous with the deficient part of the inert region of the "bb-def" X-

chromosome, it might have been expected that during the long period of existence of the *Y*-chromosome in a heterozygous state, there would have accumulated numerous lethal mutations in this part of the *Y*-chromosome. Experiments with *Y*-chromosomes from different stocks showed on the contrary that none of them exerted any lethal action in "*bb-def*" males, in spite of the fact that such males are hemizygous for a part of the *Y*-chromosome. It seems therefore highly probable that at the present time there can arise few or no gene mutations in the *Y*-chromosome of *D. melanogaster* (except that of the gene *bb*).

Furthermore, the study of the course of synapsis in *XY**Y* "*bb-def*" males suggests that the *XO* (*Protenor*) type of sex-determination can in some cases originate from the *XY* (*Lygaeus*) type, not through the loss of the *Y*-chromosome but as a result of the weakening of the synaptic affinity between the *X* and *Y*-chromosome (due, for example, to a deficiency in the region of the *X* homologous with the *Y*-chromosome). In fact, we have seen in our case that *XY**Y* "*bb-def*" males give almost exclusively *XY* and *Y* gametes. By a still greater weakening of the affinity between the *X* and *Y*-chromosomes a situation could be created where *XY**Y* males would give gametes only of these two types. On the other hand (as was shown by Stern (1929, 1930) for normal *XXYY* females, and by the present writer (unpublished) for similar females homozygous for the chromosome "*bb-def*"), such females produce only *XY* gametes. In case of a cross of such a female with an *XY**Y* male showing a greatly decreased affinity between the *X* and *Y*-chromosomes, the offspring would consist only of *XXYY* females and *XY**Y* males (Table XIV). If both the father and mother contained the same chromo-

TABLE XIV.

Eggs	Sperms	
	<i>XY</i>	<i>Y</i>
<i>XY</i>	♀ <i>XXYY</i>	♂ <i>XY</i> <i>Y</i>

somes, there would thus be established a relatively constant genetic system with an *XO* type of sex-determination (the *Y*-chromosomes imitating autosomes). The fact that the weakening of synaptic affinity between the *X* and *Y*-chromosomes leads to high non-disjunction in the male, and consequently to the appearance of *XXYY* females and *XY**Y* males, increases the possibility of the establishment of such a system.

Such a genetic system would of course be only relatively constant, inasmuch as non-disjunction both in females and in males would not as a rule lead to the formation of non-viable or sterile combinations. Never-

theless, it seems possible that such relatively constant systems may actually exist. For example, an explanation similar in principle seems to be applicable to the case of *Metapodius*, studied by Wilson (1907, 1909, 1910), where the number of Y-chromosomes varies from 0 to 6.

Lastly, the case studied illustrates one of the methods by means of which genetically inert parts of chromosomes can be inserted into genetically active parts, and shows that such a possibility should be reckoned with.

VIII. SUMMARY.

1. As a result of crossing-over between two differently inverted X-chromosomes an X-chromosome was obtained with a deficiency for a part of its genetically inert region.

2. In the XY males carrying this chromosome, the X and Y-chromosomes synapse only in 61.4 per cent. of cases. Each of the unsynapsed chromosomes is lost during disjunction approximately in one-fourth of the cases, not reaching either of the daughter nuclei.

3. XYY males carrying this chromosome produce gametes almost exclusively of the XY and Y types.

4. The case studied appears to confirm the views of Muller and Painter concerning the homology of the inert part of the X-chromosome with the Y-chromosome, and supports the opinion that few or no gene mutations can at present arise in the Y-chromosome (except those of the gene *bb*).

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THE GENETICS OF COTTON.

PART IX. FURTHER EXPERIMENTS ON THE INHERITANCE OF THE CRINKLED DWARF MUTANT OF *G. BARBADENSE* L. IN INTERSPECIFIC CROSSES AND THEIR BEARING ON THE FISHER THEORY OF DOMINANCE.

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(With Plate XI.)

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INTRODUCTION.

IN a previous paper (1932 *a*) the writer presented the results of experiments on the mode of inheritance of the Crinkled Dwarf mutant of *G. barbadense* L. when crossed with *G. hirsutum* L. It was shown that the F_1 of such a cross, instead of exhibiting complete dominance of normal, showed distinct crinkling, and the crinkling persisted in successive back-crosses of heterozygote to normal *G. hirsutum* L.

These experiments were carried to the fourth back-cross, in which (*a*) the ratio of 1 crinkled (intermediate) : 1 normal was obtained, (*b*) the crinkled class was practically uniform, (*c*) the normals were completely *hirsutum* in type, with no trace of *barbadense* genes.

For the purpose of the Fisher theory it was important to observe the characters of both homozygous and heterozygous crinkled when trans-

ference to *hirsutum* was complete. Comparison was therefore made of the selfed offspring of both fourth and sixth back-cross heterozygotes. It was also important to study the behaviour of crinkled when transferred to other types of *hirsutum*.

THE EXPERIMENTAL RESULTS.

The fourth back-cross selfed.

The *hirsutum* to which the heterozygote was continuously back-crossed was a variety of Upland known as Meade, and in our records as Type 9. It has been selfed for at least seven generations and is probably almost a pure line.

Selfing of the heterozygous fourth back-cross plants resulted in segregation into the three expected classes, normal, intermediate crinkled, and extreme crinkled. The results are presented in Table I below.

TABLE I.

*Results of selfing fourth back-cross heterozygotes of cross
barbadense crinkled × hirsutum normal.*

Family	Normal	Intermediate	Crinkled	Total	Seeds sown
351	2	4	3	9	13
352	6	17	15	38	44
353	0	9	4	13	21
356	1	5	2	8	19
357	5	4	5	14	21
358	4	10	5	19	27
359	9	14	8	31	36
Total	27	63	42	132	181
<i>Expected</i>	33	66	33		
<i>Expected on No. seeds sown</i>	45	90	45		

Here it will be seen that a close approximation to a 1:2:1 ratio is obtained. There is, however, a deficiency of normals and heterozygotes as compared with crinkled, since crinkleds show nearly perfect agreement with expectation on number of seeds sown (93 per cent.), the heterozygote class coming next (70 per cent.), and the normal last (60 per cent.).

General characters of homozygous and heterozygous hirsutum crinkled.

The crinkled class was uniform. Under field conditions they grew to a height of about 9 in. compared with 2-3 ft. in the intermediate and normal classes. Comparison with *barbadense* crinkled in respect of vigour showed that on the whole they were much less vigorous and less able to survive under unfavourable field conditions. No plant produced more

than two bolls, whereas *barbadense* crinkled has been observed to produce three or four times this number. Nevertheless, when given careful culture in the greenhouse, there is much less difference in the vigour of the two types, and *hirsutum* crinkled is not noticeably at a disadvantage. The phenotypic characters of *barbadense* crinkled, *hirsutum* crinkled, and *hirsutum* heterozygote are illustrated in Plate XI.

*Productivity of hirsutum heterozygotes compared with normals
(fourth back-cross selfed).*

In view of Fisher's assumption (1928)—necessary to his hypothesis of the origin of dominance—that on the initial appearance of a mutant in a species the heterozygote would be at a disadvantage compared with the wild type, observations were made on the productivity of *hirsutum* heterozygotes compared with normals. The results of the examination for number of bolls per plant are placed below in Table II.

TABLE II.

Number of bolls per plant of hirsutum heterozygotes and normals.

Family		5	10	15	20	25	30	35	40	Mean	Condition
G 351	N.	.	.	1	1	17.5	Good
	Cr.	.	.	2	1	.	1	.	.	20.0	
G 352	N.	.	.	1	2	1	2	.	.	23.3	Good
	Cr.	.	.	2	4	5	4	.	2	25.6	
G 353	N.	No normals									Bad
	Cr.	5	4	7.2	
G 356	N.	.	.	1	15.0	Good
	Cr.	.	1	.	2	1	1	.	.	21.0	
G 357	N.	3	2	7.0	Bad
	Cr.	1	3	8.8	
G 358	N.	2	2	7.5	Bad
	Cr.	7	2	1	7.0	
G 359	N.	4	3	1	1	9.4	Medium
	Cr.	7	5	2	8.2	
Total	N.	9	7	4	4	1	2	.	.	12.6	
	Cr.	20	15	7	7	6	6	.	2	14.4	

It may be noted that:

(1) In the most productive families (G 351 and G 352) there is a slight but definite *increase* in number of bolls per plant in the heterozygous class as compared with the normal.

(2) In families where the yield is low, through bad conditions, there is practically no difference in the yields of the two classes. Bad soil conditions, however, tend to emphasise the crinkledness and to reduce the size of the leaves.

(3) The summarised results show that on a *hirsutum* (Type 9) genotype background, heterozygous crinkled is not appreciably at a disadvantage compared with normal even under bad conditions, while under good conditions it may actually be at some advantage.

Second generation from fourth back-cross selfed.

Normals—twelve families from normals gave normals only.

Intermediate crinkled—ten families from intermediate crinkleds were grown, and all segregated into the expected three classes, viz.: normal, intermediate crinkled, and crinkled. The results follow in Table III.

TABLE III.

Second generation from fourth back-cross selfed.

Family	Progeny of intermediates			No. of seeds sown
	Normal	Intermediate	Crinkled	
302	4	4	2	104
332	1	2	1	83
341	2	7	5	45
349	7	16	7	78
353	3	6	3	20
361	6	7	2	56
363	5	15	7	92
375	18	30	10	153
376	13	30	10	170
377	12	31	12	261
Total	71	148	59	
<i>Expected</i>	<i>69.5</i>	<i>139.0</i>	<i>69.5</i>	

It will be seen that the ratio of the three phenotypes is not appreciably different from the expected 1 : 2 : 1 ratio. It was hoped that the results might throw some light on the excess of crinkled reported in Table II, but the germination of the seed was very bad, and it was not possible to examine the plants in the field till they were several weeks old. The results cannot therefore provide any indication of the proportions of the three types present in the original seed.

The productivity of the three types was not recorded, though the intermediates seemed to be fully as productive as the normals.

Crinkled. Three families were grown and all bred true to crinkled of uniform type.

The sixth back-cross selfed.

In order to see whether any change in *hirsutum* crinkled would result from further back-crossing to Type 9, several sixth back-cross heterozygotes were selfed. The results follow in Table IV.

TABLE IV.

Results from selfing heterozygotes of sixth back-cross.

Family	Normal	Inter- mediate	Crinkled	Total	Seeds sown
G 1823	10	21	9	40	48
G 1824	6	13	9	28	31
G 1825	10	25	11	46	48
G 1826	18	17	17	52	52
G 1827	5	14	12	31	36
G 1828	4	13	14	31	38
Total	53	103	72	228	258
<i>Expected on 1:2:1 basis</i>	57	114	57		
<i>Expected on seeds sown</i>	64.5	129.0	64.5		

As in the progeny of the fourth back-cross there is a deficiency of normal as compared with crinkled, and although it is clear that under field conditions crinkled is at considerable disadvantage, it is possible that during the period of inter-ovular competition crinkled may be at an advantage.

It was again observed that the degree of crinkledness varied considerably with environmental conditions. Family 1826, which was kept in the greenhouse in 6 in. pots under optimum cultural conditions, showed little difference between normal and heterozygote until about 5 weeks old when separation became practicable. Other families planted at the same time in the field showed early development of crinkling in heterozygotes.

Comparison of height of normals and heterozygotes of sixth back-cross selfed.

Family 1826 (see above) was grown in 6 in. pots under good cultural conditions, and the height of the normal and intermediate groups measured at 5 weeks old. The results follow in Table V.

TABLE V.

Frequency array of height of normals and intermediates of sixth back-cross selfed. Plants 5 weeks old.

	Height in cm.									Mean
	18	19	20	21	22	23	24	25	26	
Normal	.	.	3	1	1	4	.	.	.	21.7
Intermediate	1	1	3	1	3	1	5	.	1	22.1

Here it will be seen that the heterozygotes are slightly taller though more variable than the normals. On the whole the results confirm those previously put forward in connection with the selfed fourth back-cross,

viz. that the heterozygote under good conditions may be at an advantage compared with normal.

Counts were made of the number of bolls per plant of the remaining families planted in the field. The results are presented in Table VI.

TABLE VI.

Frequency arrays of No. of bolls per plant of normal, intermediate and crinkled in selfed sixth back-cross.

		No. of bolls per plant																						
Family		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Mean
G 1823 S.	N.	1	2	1	2	2	1	.	.	.	1	10.8
	Int.	1	1	.	.	1	2	4	1	3	2	2	.	1	.	.	1	13.1
	Cr.	.	.	7	1	2	1	2.7
G 1824 S.	N.	2	.	1	.	.	.	1	1	1	12.8
	Int.	1	1	1	2	1	2	.	.	3	.	.	1	.	1	.	.	.	10.4
	Cr.	.	.	2	.	4	1	2	4.1
G 1825 S.	N.	1	1	3	.	1	.	.	.	2	2	12.1
	Int.	.	.	1	.	.	.	1	.	1	6	2	3	.	1	2	3	2	.	2	.	1	.	11.9
	Cr.	3	4	2	2	1.3
G 1827 S.	N.	.	1	1	1	.	.	1	.	.	.	1	8.4
	Int.	.	.	.	1	.	.	1	2	1	1	1	1	.	1	.	.	3	1	1	1	.	.	11.7
	Cr.	.	.	1	.	5	3	1	2	5.6
G 1828 S.	N.	1	1	1	1	12.5
	Int.	.	.	.	3	.	2	.	.	1	.	1	.	1	2	1	.	2	.	.	.	1	.	10.1
	Cr.	1	3	7	1	2	2.0
Total	N.	.	1	.	.	1	.	.	1	2	5	4	5	4	2	1	4	3	2	11.4
	Int.	.	.	1	4	.	3	4	4	5	8	7	6	5	8	6	5	10	1	5	1	2	1	11.6
	Cr.	4	7	19	4	13	5	3	2	2.9

The above results again go to show that the yield of the heterozygote is slightly above that of the normal. The yield of the crinkled plants is very low and is in accordance with the great reduction which they exhibit in height and in the size of all the vegetative parts of the plant. To sum up: The transference of a taxonomically restricted mutant, (crinkled) from a species, *G. barbadense* L. in which it is common, to a variety (Type 9) of another, *G. hirsutum* L. in which it is unknown, results in a change in ratio in the progeny of heterozygotes from 3 normal: 1 crinkled, to a ratio of 1 normal: 2 intermediate: 1 crinkled. Further, there is evidence that the heterozygote when so transferred is somewhat more productive than the normal under good conditions.

TRANSDUCENCE OF *hirsutum* CRINKLED TO TWO OTHER VARIETIES OF *G. hirsutum*.

It was recognised that the results of the experiments just described could be recognised as valid only for the particular variety of *G. hirsutum* grown, and that in order to obtain general confirmation of the conclusion

it was necessary to transfer crinkled to the genetic background characteristic of other types of *G. hirsutum*. Experiments were accordingly initiated with this object in view.

(1) *To G. hirsutum (variety Virescent Yellow).*

A heterozygous crinkled of the fourth back-cross was crossed with the Upland variety Virescent Yellow. This variety differs somewhat from T 9, but only in such minor characters as length of lint, boll size, etc. In the fifth back-cross seven plants were obtained, four normal and three crinkled. The development of the crinkled character was rather less intense than in the corresponding T 9 heterozygote, and one of the plants was so slightly crinkled that there was at first some doubt respecting its classification.

To obtain the sixth back-cross all three heterozygotes were used and three families obtained. *It proved impossible to classify the plants into normal and heterozygous crinkled. Most of the plants were typically normal, but a few showed faint crinkling of the young leaves.* Selfed families were grown from nine plants, and the results are set out in Table VII.

TABLE VII.

Results of selfing sixth back-cross heterozygotes of cross barbadense crinkled × hirsutum normal (variety Virescent Yellow).

	Normal	Intermediate	Crinkled	Remarks
G 1161 × G 1145				
G 2168	14	—	—	True to normal
G 2169	15	—	—	True to normal
G 2172	3	2	3	Intermediates phenotypically near to normal
G 2173	11	—	4	Clear segregation into 3 normal : 1 crinkled
G 1163 × G 1145				
G 2124	10	2	5	1 intermediate similar to T 9 intermediate
G 2125	7	—	4	Clear segregation into normal and crinkled
G 1158 × G 1145				
G 2178	16	—	—	True to normal
G 2179	17	—	—	True to normal
G 2180	2	1?	2	1 very doubtful intermediate crinkled

In segregating families even the slightly crinkled class is seen to be rare and crinkling of the degree found in the T 9 heterozygote is only found in one plant out of five. Two families show a clear segregation into 3 normal : 1 crinkled.

These results show unequivocally that in at least one typical member of the *hirsutum* group there may be *complete* dominance of normal over crinkled.

(2) To *G. hirsutum* (variety *Triumph T 57*).

An extracted crinkled from fourth back-cross selfed was crossed with a single plant of T 57. Six F_1 plants were grown and it was observed that none of the plants was as crinkled as the T 9 heterozygote. Faint traces of crinkling were seen in two plants which were selfed, giving the following results:

Family	Normal	Slightly crinkled	Crinkled	Remarks
G 1584	22	10	6	Most intermediates were very near normal
G 1585	10	4	2	Most intermediates were very near normal

The slightly crinkled group contained no plants as crinkled as the T 9 heterozygotes, and many on a cursory examination would be classified as normal. As in the previous experiment there can be no doubt that the normal group contains a mixture of normals and heterozygotes, and that T 57 contains a modifier complex which may also confer dominance upon normal.

Plant G 1584 (heterozygote) was crossed again with T 57 and gave 20 plants. All these were normal except two, which exhibited faint traces of crinkling. Definite proof is thus provided that the *hirsutum* variety T 57 is as dominant over its own particular crinkled as the corresponding *barbadense* normal is over the *barbadense* crinkled.

DISCUSSION.

In the writer's previous paper (1932 *a*) on the mode of inheritance of crinkled in the interspecific cross *barbadense* \times *hirsutum*, it was stated that the experimental evidence was fully in accordance with expectation on Fisher's theory (*loc. cit.*) that dominance of the wild type is due to the reaction of the heterozygote to modifying factors which ultimately cause it to be indistinguishable from the wild type. It was, however, considered an improbable assumption that normals descended from heterozygotes had replaced the original normal population.

In the first part of this paper it is shown that after four back-crosses of heterozygous crinkled to *hirsutum* (T 9) two sharply demarcated classes are produced, intermediate crinkled heterozygotes and normals. The normals breed true while the heterozygotes segregate in the ratio 1

normal : 2 intermediate : 1 crinkled. Two further back-crosses of heterozygote to normal Upland resulted in no change in the phenotypic appearance of the heterozygote, and the behaviour of the sixth back-cross on selfing differed in no respect from that of the fourth back-cross.

So far the experimental evidence is fully in accordance with the Fisher theory of dominance.

The theory, however, in its original form breaks down completely in the light of new evidence obtained through experiments to transfer crinkled to other strains of *G. hirsutum*. It is shown that there is *complete dominance* of normal over crinkled in the genotypic background of the variety *Virescent Yellow*, and also in that of a pure line derived from the well-known commercial Upland variety *Triumph*. It is possible that the relation 3 normal : 1 crinkled holds good generally throughout the species *G. hirsutum*, and that the ratio 1 normal : 2 intermediate : 1 crinkled found in T 9 is exceptional.

The variety which exhibits the 1 : 2 : 1 ratio (Type 9) is a pure line extracted from the commercial variety Meade, which in most morphological characters differs very little from ordinary standard varieties of Upland. It possesses much longer and finer staple than Upland and is alleged to be descended from a cross between Sea Island (*barbadense*) and Upland, and to derive the long lint from a Sea Island progenitor. Since according to our experiments long lint is not simply inherited but is the result of multiple factors, it is likely that Meade may have a certain number of *barbadense* genes, which two back-crosses with the varieties *Virescent Yellow* or T 57 are adequate almost completely to displace, but which nevertheless show their presence by a genotypic effect on the normal dominance reaction system of *G. hirsutum*.

It was noted from the results of the first back-cross, (*barbadense* crinkled \times *hirsutum* normal) \times *hirsutum* normal (*loc. cit.*), that it was possible to obtain a heterozygous crinkled much nearer to the homozygous form than the ultimate *hirsutum* crinkled obtained after several back-crosses.

It is clear that *hirsutum* and *barbadense* have a markedly different make-up of the association of genes which constitutes the character "normal" as opposed to the character "crinkled."

This is shown best by the fact of blending inheritance between *barbadense* crinkled and *hirsutum* normal. This has been demonstrated to occur in the F_2 of crosses between *hirsutum* T 57 and *barbadense* crinkled, as well as in several other crosses in which different *hirsutum* parents were used.

When the two sets of dominance modifiers are brought into contact, there will occur an inability to preserve the characteristic species dominance mechanism, since each species will presumably contain allelomorphs of the dominance modifiers of the other.

It cannot now be assumed that the occurrence of a mutant in a species is necessary for the acquisition of dominance by the wild type, and the fact that *hirsutum* in two types at least is protected against mutation in the crinkled locus, coupled with the further observation that the crinkled mutant does not occur at all in this species, renders necessary some modification of the Fisher theory.

The view arrived at as a result of these experiments may be briefly stated.

Though the point of view emphasised by Fisher that dominance is a function of the genotype as a whole is thoroughly substantiated, the attainment in a species of dominance over a deleterious mutation may be quite unconnected with modification of the reaction of the species to the mutant through the occurrence of initially disadvantageous heterozygotes. It is apparently attained by the species, in this particular instance at least, irrespective of the occurrence of the mutant, and it must be assumed that protection against deleterious recessives is arrived at because the modifiers of dominance are of advantage to the wild type, and are thus selected on their own account.

The suggestion has been put forward by Haldane (1930) that one possible explanation of the behaviour of crinkled dwarf in interspecific crosses is that the New World cottons are tetraploids, and that failures of Mendelian inheritance are to be expected on crossing them.

A survey of the genetic data presented by the writer (1932 *b* and previous papers) indicates that all the complications encountered are explicable on the basis of differences in modifier complexes characteristic of the different species which yield to straightforward analysis by studying the behaviour of a gene on a constant genetic background—obtained by back-crossing to the requisite genotype and then selfing. Granted that the New World cottons are polyploids, they exhibit no signs of this in their genetic behaviour, with the exception that cases of duplicate genes exhibiting normal 15 : 1 F_2 ratios have been several times encountered.

SUMMARY.

1. Further experiments are described on the mode of inheritance of the crinkled dwarf mutant of *G. barbadense* Linn. when crossed with normal *G. hirsutum* Linn.



Fig. 1.

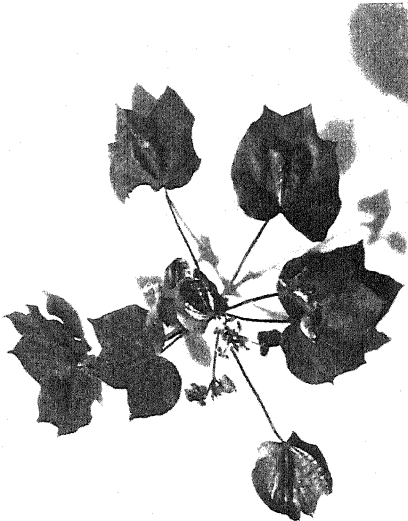


Fig. 2.



Fig. 3.

2. Observations were made on the characters of crinkled when transferred by repeated back-crossing to *G. hirsutum* (T 9). Selfing of the heterozygotes of the fourth back-cross plants produced normal, intermediate crinkled, and extreme crinkled in a 1 : 2 : 1 ratio, and the results from selfing six back-cross heterozygotes showed that no change had taken place through further back-crossing.

3. The new type of *hirsutum* crinkled was apparently slightly less vigorous and productive than the original *barbadense* mutant, though under good conditions little difference was observable.

4. *Hirsutum* heterozygous for the crinkled factor was shown to have a slight advantage over normal under good conditions and was not at any considerable disadvantage under bad conditions.

5. Transference of crinkled to two further types of *hirsutum* revealed complete or nearly complete dominance of *hirsutum* to the crinkled type.

6. The bearing of the experiments on Fisher's theory of dominance is discussed and it is concluded that modification of the theory is necessary. Complete dominance of normal over crinkled exists in two types of *G. hirsutum* although the crinkled mutant does not occur in that species. It is concluded that modifiers of dominance are of advantage to the wild type and are thus selected on their own account.

EXPLANATION OF PLATE XI.

Fig. 1. Crinkled from Sea Island variety of *G. barbadense* L.

Fig. 2. Extracted homozygous crinkled from back-cross of (*barbadense* crinkled \times *hirsutum* T 9 normal) \times *hirsutum* T 9 normal. Sixth back-cross selfed.

Fig. 3. Heterozygous crinkled showing intermediate type of crinkling in a seventh back-cross heterozygote (*G. hirsutum* T 9).

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STUDIES IN *PRUNUS*, IV

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(With Three Text-figures.)

IN a first account of chromosome behaviour in *Prunus* (Darlington, 1928) I stated that the domesticated sweet cherry varieties had one, two, or three chromosomes in excess of the typical diploid number of 16 found in the species *Prunus avium*. This conclusion was derived from observations of side views of the first metaphase of meiosis. In these, tripartite structures were seen which I interpreted as trivalents, although allowing that a bivalent "can only be distinguished from a trivalent by the usually stronger connection between the three bodies." In polar view eight bodies were seen and study from this aspect gives, as Kobel (1927) states, no suggestion of abnormal configurations. When later I was able to



Figs. 1-3. Metaphases in the root tip.

Fig. 1. Governor Wood. Fig. 2. Black Eagle. Fig. 3. Waterloo. $\times 3000$.

interpret the bivalent configurations more exactly in terms of chiasmata (*i.e.* of exchanges of partner amongst the chromatids) I found that the various forms of supposed trivalent could be interpreted equally satisfactorily as bivalents with interstitial chiasmata (Darlington, 1930). Somatic mitoses in the flower buds were unsatisfactory. In order therefore to resolve the question finally it was desirable to study root tips of the grafted cherry varieties themselves. These have not been available in the past owing to the difficulty of rooting cherries. My colleague, Mr M. B. Crane, has, however, succeeded in obtaining roots for me by a special method. The tree was planted horizontally, and as lateral shoots developed they were earthed up and young roots appeared at the callused nodes.

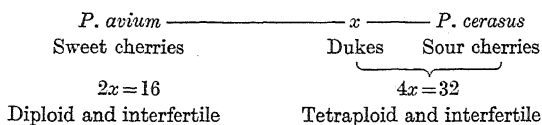
The result of the study of these roots is to show, in three of the most important varieties, that the somatic number is 16 (Figs. 1-3). There

seems little doubt that all the other sweet cherries are similarly diploid, since the same type of evidence was used in all cases.

An important consequence arises from this. It can no longer be supposed that the three varieties, raised by Knight between 1814 and 1818, Knight's Early Black, Black Eagle and Waterloo, are indeed the result of crosses with pollen of May Duke, as Knight stated them to be, for May Duke is a tetraploid ($2n=32$) which on no analogy could be supposed to produce the necessary haploid ($n=8$) pollen. It must be concluded rather that the three varieties are of purely sweet cherry origin and that the sour cherry characters they show are derived from the range of variation of their own diploid species.

The influence of the sweet cherry on the sour remains, however, indisputable, for, as described earlier, tetraploid seedlings appear amongst the progenies of diploid sweet cherries, as well as amongst their crosses with sour cherries, through the failure of meiosis and the production of unreduced gametes. The latter class corresponds with the Duke cherries in habit as well as in chromosome number, and the Dukes cross freely with the sour cherries (Crane and Lawrence, 1929).

The fertile relations of the two species may therefore be described diagrammatically as follows (the normal triploid hybrids being sterile):



SUMMARY.

The sweet cherries (*Prunus avium*), which were previously reported to have extra chromosomes, are found from root tips to be diploid ($2x=16$). They cannot therefore be derived directly from hybridisation with sour cherries, as hitherto believed.

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STUDIES ON NON-INHERITED VARIATION IN
CROSSING-OVER IN *DROSOPHILA*.

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(With Seven Text-figures.)

I. INTRODUCTION.

THE causes which produce variation in crossing-over in *Drosophila* may roughly be classified into the following two categories: (a) inherited or permanent causes, e.g. sexual difference, chromosomal aberrations, gene modifiers; (b) non-inherited or temporary causes, e.g. high or low temperature, X-ray or radium irradiations, temporarily enforced sterility of adult female, prolongation of the larval and pupal stages. The age of the female may be also included in this latter category for the reason to be described later.

Under the influence of the former causes, the flies show an abnormal cross-over value throughout their lives, i.e. the effect is permanent, while under the influence of the latter causes, they show an abnormal cross-over value only as long as the causes or the influence of the causes exist, i.e. the effect is temporary. This paper deals with the latter cases only. The preliminary report of this investigation was published in 1931.

II. BASIC PROPERTIES CONCERNING THE TEMPORARY
VARIATION IN CROSSING-OVER.

From my own experimental results obtained in both *D. virilis* and *D. melanogaster*, and also from the data given by a number of previous investigators on *D. melanogaster*, the following two conclusions have been established. The first is that the cross-over frequency has a tendency to decrease in the distal region, if it increases in the proximal region (near the spindle-fibre attachment), and *vice versa*. Stated in another way, the phenomenon of "compensation," according to Muller's (1926) terminology, seems to hold in any case of induced variation in crossing-over.

The second property is that the after-effect of any stimulus (e.g. X-ray irradiations, abnormal temperature) on crossing-over appears in the form of a wavy curve.

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(1) *Compensatory relation of the cross-over frequency.*

The preliminary experiment relating to the first property was carried out by using *D. virilis* as material, and the effect of high temperature on the cross-over frequency in the first chromosome was examined. For this purpose, the three regions, *yellow-crossveinless*, *darky-white* and *ragged-bobbed*¹ were selected as the distal, intermediate and proximal region respectively (Table I). Each experiment was performed under

TABLE I.

Principal genes and their loci in the X-chromosome of Drosophila virilis
(Chino, 1929, 1930, and his unpublished data).

Gene	Locus	Character
<i>Yellow (y)</i>	3.0	Body colour
<i>Echinus (ec)</i>	8.5	Eye surface
<i>Vermilion (v)</i>	24.5	Eye colour
<i>Crossveinless (cv)</i>	26.0	Crossveins
<i>Singed (sg)</i>	51.0	Bristles
<i>Darky (dy)</i>	78.1	Wing shape
<i>White (w)</i>	102.0	Eye colour
<i>Apricot (ap)</i>	130.0	Eye colour
<i>Ragged (rg)</i>	157.0	Wing margin
<i>Bobbed (bb)</i>	168.0	Bristles and abdomens

similar conditions; namely, the larvae carrying the genes in the heterozygous state, were divided into two groups, and were bred at 30° C. and 25° C. respectively. After their emergence, the females only were selected and mated to males having the necessary recessive characters, and were then bred, as before, at 30° C. and 25° C. respectively. In the *ragged-bobbed* series, only the females were counted, since the males do not show the *bobbed* character phenotypically. The result is given in Table II.

TABLE II.

Recombination percentage at 25° C. and 30° C. in the distal, intermediate and proximal regions of the X-chromosome of D. virilis.

	Distal (y-cv)		Intermediate (dy-w)		Proximal (rg-bb)	
	R.V.	Total	R.V.	Total	R.V.	Total
30° C.	19.06 ± 1.44	745	23.91 ± 1.90	506	19.67 ± 1.73	514
25° C.	28.93 ± 1.73	674	26.09 ± 1.58	774	16.18 ± 1.72	468
Diff./σ	9.87/2.25 = 4.4		2.18/2.47 = 0.9		3.49/2.43 = 1.4	

In the table, it is of interest that the cross-over value of the distal region was decreased markedly by high temperature. The same tendency

¹ The fact that *ragged* and *bobbed* genes are located near the proximal portion of the X-chromosome was confirmed by the data concerning equational exceptions, and also by the chromosomal configurations found in several translocations involving the X-chromosome (Kikkawa, 1932 b, and unpublished data).

seems to be found in the intermediate region, but in a very slight degree. On the other hand, the cross-over value of the proximal region is increased by high temperature. This compensatory relation was confirmed more definitely by the data concerning the variation in crossing-over in relation to age of females, and also by further data on high temperature (Tables III, IV, Fig. 1). In these experiments, three recessive genes, *echinus*, *vermilion* and *singed*, were taken from the distal region, and three other genes, *apricot*, *ragged* and *bobbed*, were taken from the proximal region. The experiments were carried out by a method similar to that employed by Plough (1917, 1921), Bridges (1927, 1929) and the present author

TABLE III.

Variation in crossing-over at 25° C. and 30° C. in the distal regions of the X-chromosome of *D. virilis*.

	$\frac{ec, v, sg}{+, +, +} \times ec, v, sg.$			
Days after emergence	Regions		Coincidence	Total number of flies
	(<i>ec-v</i>)	(<i>v-sg</i>)		
	Emergence at 30° C., bred at 25° C.			
4-8	6.46	17.91	0.77	681
9-13	9.76	19.01	0.73	784
14-18	16.27	19.25	0.45	639
19-23	22.69	25.37	0.69	335
24-28	18.86	24.12	0.57	228
29-33	19.01	23.24	0.80	142
	Emergence and bred at 25° C.			
4-8	16.48	20.27	0.74	1486
9-13	20.41	23.20	0.67	1039
14-18	20.81	21.75	0.66	639
19-23	19.74	20.62	0.43	854
24-28	24.01	22.30	0.57	583
29-33	23.30	25.47	0.52	369

(*loc. cit.*). Briefly stated, two groups of females carrying the genes in a heterozygous state, which emerged within a day at 30° C. and 25° C., were kept for the first 3 days in a mass culture with males having the necessary genes (during this period, the females do not generally lay eggs), and then each female, either in the treated or control series, was transferred to a fresh vial every fifth day, and was bred at 25° C. continuously. In Table IV only the females were counted, since the males, as stated before, do not show the *bobbed* character phenotypically.

The results of Tables III and IV show that the region which is affected by high temperature is practically restricted to the portions near the distal and proximal ends of the genetic map, and the effect is largely limited to the region very near the proximal end. Further, if we take the

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region between *echinus* and *vermilion*, and that between *ragged* and *bobbed*, and plot the curves of recombination value for each period, we notice that the age variation in crossing-over also shows the phenomenon of compensation (Fig. 1).

TABLE IV.

Variation in crossing-over at 25° C. and 30° C. in the proximal regions of the X-chromosome of *D. virilis*.

	$\frac{ap, rg, bb}{+, +, +} \times ap, rg, bb.$			
Days after emergence	Regions		Coincidence	Total number of flies
	(<i>ap-rg</i>)	(<i>rg-bb</i>)		
Emergenced at 30° C., bred at 25° C.				
4-8	22-21	15-76	0-61	698
9-13	22-31	8-52	0-62	928
14-18	22-43	12-71	0-52	1070
19-23	23-95	9-67	0-34	1148
24-28	22-69	7-67	0-30	952
29-33	21-28	9-41	0-65	691
Emergenced and bred at 25° C.				
4-8	22-98	11-11	0-74	792
9-13	21-89	9-19	0-65	1142
14-18	22-97	7-68	0-29	1036
19-23	21-67	8-25	0-30	946
24-28	22-46	6-58	0-37	730
29-33	23-17	6-03	0-34	846

The frequency of recombination at 30° C. in this table is less than that of control in Table I (25° C.). This fact, that seems to be a contradiction, is probably due to the stocks used in these experiments. Because, in *D. virilis*, the frequency of crossing-over is variable according to the strain of the material.

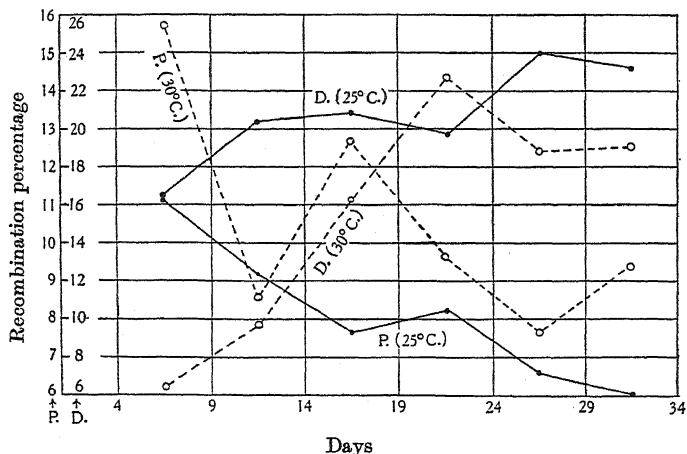


Fig. 1. Curve showing the percentage of recombination for the *ec-v* and the *rg-bb* region in the X-chromosome of *D. virilis* at 25° C. and 30° C. P. (30° C.), proximal region (*rg-bb*) at 30° C. D. (30° C.), distal region (*ec-v*) at 30° C., etc. (Tables III and IV.)

Further evidence for this is given by a similar experiment on material involving *yellow* and *vermilion* in the distal region of the same chromosome (Table V, Fig. 2). Experiments on the autosome of this species

TABLE V.

Variation in crossing-over in the yellow-vermilion region of the X-chromosome of *D. virilis*.

$$\frac{(se), (sc), y, v, (cv)}{(+), (+), +, +, (+)} \times (se), (sc), y, v, (cv).$$

Days after emergence	Series I (Emerged and bred at 25° C.)		Series II (Emerged at 30° C., bred at 25° C.)		Series III (Emerged and bred at 25° C.; exposed to 30° C., at 9th-12th day)	
	R.v.	Total	R.v.	Total	R.v.	Total
4-8	28.00	150	18.25	126	29.23	585
9-12	30.37	820	23.09	533	29.62	989
13-15	28.35	836	27.31	659	26.90	855
16-18	25.91	629	30.59	729	24.54	489
19-21	25.43	586	28.67	851	19.19	323
22-24	26.24	442	25.00	864	25.47	267
25-27	28.65	370	28.67	678	26.22	225
28-30	24.85	173	22.88	555	30.72	186

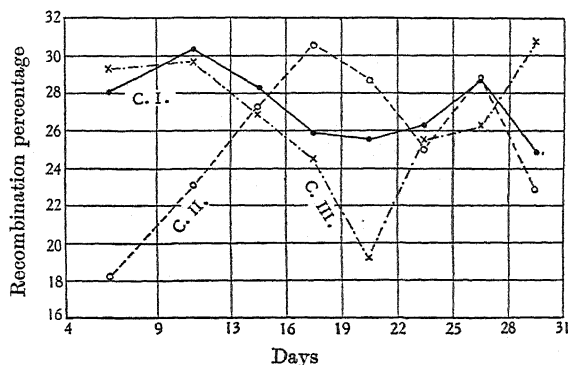


Fig. 2. Curves showing the variation in recombination percentage for the *y-v* region in the X-chromosome of *D. virilis* at 25° C. and 30° C. C.I., the control curve (emerged and bred at 25° C.). C.II., the curve in the case where the females were emerged at 30° C., and bred at 25° C. C.III., the curve in the case where the females were emerged and bred at 25° C., except during the period 9th-12th day, when they were exposed to 30° C. (Table V.)

yield very similar results. For example, the cross-over frequency between *ruffled* and *scarlet*, and that between *scarlet* and *eosinoid* (these are all located in the distal portion of the fifth chromosome of *D. virilis*) are decreased markedly by either high temperature (Table VI), or X-ray irradiation (Table VII).

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Fujii's (1933) results on the effect of X-rays on the cross-over frequency in the first chromosome of *D. virilis* are quite similar to those on the effect of high temperature stated above. Thus it seems that this phenomenon of compensation in the cross-over frequency is of universal occurrence, at any rate in *D. virilis*.

It seems to hold also for *D. melanogaster*, for in the X-chromosome of this species, Mavor (1923 *a, b*) has pointed out that X-ray irradiations

TABLE VI.

Recombination percentage at 25° C. and 30° C. in the distal regions of the fifth chromosome of D. virilis.

	$\frac{ru, sr, es}{+, +, +} \times ru, sr, es.$			
	Regions			
Temperature	(<i>ru-sr</i>)	(<i>sr-es</i>)	Coincidence	Total
30° C.	14.45 ± 0.53	16.27 ± 0.55	0.32	2035
25° C.	20.86 ± 0.66	19.08 ± 0.67	0.32	1745
Diff./σ	6.41/0.84 = 7.6	2.81/0.87 = 3.2	—	—

TABLE VII.

Effect of the X-ray irradiations on the cross-over frequency in the distal regions of the fifth chromosome of D. virilis.

Dosage: Coolidge tube with tungsten target, 50 kV, 4 ma, 21 cm., 30 min., non-filter.
Count was done by Muller's (1925) method.

	$\frac{ru, sr, es}{+, +, +} \times ru, sr, es.$			
	Regions			
Kinds	(<i>ru-sr</i>)	(<i>sr-es</i>)	Coincidence	Total
X-rayed	16.16 ± 0.82	16.92 ± 0.84	0.59	916
Control	21.22 ± 0.81	19.39 ± 0.78	0.46	1160
Diff./σ	5.06/1.15 = 4.4	2.47/1.15 = 2.2	—	—

cause a marked decrease in crossing-over for the *eosin-miniature* region. Muller (1926) reports that X-rays may produce a slight decrease in crossing-over for the distal region (*scute-apricot*), but an increase for the proximal region (*Bar-Beadex*). Stern (1926) states that high temperature produces an increase in crossing-over for the proximal region (*Bar-bobbed*), and also that this region shows a slight variation in crossing-over with age of the females.

Hitherto it has been believed that neither age nor alteration in temperature produce any significant change in crossing-over for the distal part of the X-chromosome (Bridges, 1915; Plough, 1917, 1921). To re-examine this point more crucially, a series of experiments shown in

Table VIII and Fig. 3 was performed. Females of the constitution of *yellow* (0.0), *crossveinless* (13.7) and *vermilion* (33.0), and the males of Oregon-wild stock were used in the first mating. The method was the

TABLE VIII.

Variation in crossing-over at 25° C. and 31° C. in the first chromosome of *D. melanogaster*.

	$\frac{y, cv, v}{+, +, +} \times y, cv, v.$			
Days after emergence	Regions		Coincidence	Total number of flies
	(<i>y-cv</i>)	(<i>cv-v</i>)		
Emerged at 31° C., bred at 25° C.				
3-4	14.7	19.5	0.55	421
5-6	17.8	23.3	0.43	433
7-8	20.6	24.8	0.68	664
9-10	21.0	23.0	0.57	653
11-12	20.1	24.1	0.33	622
13-14	19.3	23.6	0.32	622
15-16	20.8	27.7	0.47	487
Emerged and bred at 25° C.				
3-4	16.3	23.3	0.42	397
5-6	22.2	23.8	0.42	492
7-8	19.3	22.6	0.38	535
9-10	20.1	24.0	0.18	562
11-12	19.8	25.7	0.32	610
13-14	17.0	25.6	0.24	577
15-16	20.4	21.4	0.18	509

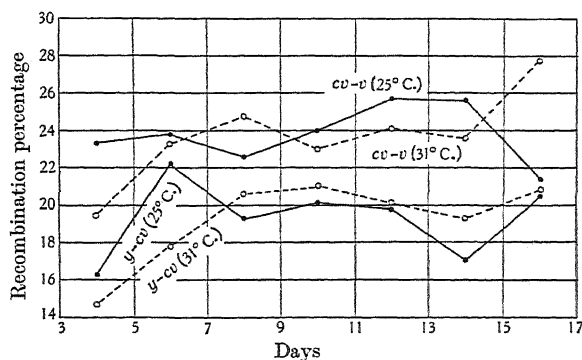


Fig. 3. Curves showing the percentage of recombination for the *y-v* and the *cv-v* region in the X-chromosome of *D. melanogaster* at 25° C. and 31° C. (exposed to this temperature at only larval and pupal stages). (Table VIII.)

same as that employed in the preceding experiments. Although the number of flies used in this experiment was small, the result leaves little doubt that high temperature causes a slight decrease in crossing-over for the distal region, and further that the region shows a slight variation in crossing-over with age of the females. It is of great interest to compare

the curves in Fig. 3 with those obtained by Stern (1926) for the proximal region (*Bar-bobbed*). The compensatory relation in the cross-over frequency is found both in the data on high temperature, and in those on the age

TABLE IX.

Variation in crossing-over at 25° C. and 31° C. in the second chromosome of D. melanogaster.

$$\frac{b, pr, (c), px, sp}{+, +, (+), +, +} \times b, pr, (c), px, sp.$$

Days after emergence	Regions			Total number of flies
	(<i>b-pr</i>)	(<i>pr-px</i>)	(<i>px-sp</i>)	
	Emerg. at 31° C., bred at 25° C.			
3-4	16.1	41.4	5.1	573
5-6	13.2	37.4	8.4	393
7-8	17.5	44.3	6.3	348
9-10	5.8	45.3	7.6	276
11-12	4.2	43.9	4.9	264
13-14	3.3	38.0	9.2	183
15-16	0.7	38.0	8.0	137
Emerg. and bred at 25° C.				
3-4	5.7	44.6	9.3	525
5-6	3.2	45.2	7.2	502
7-8	4.5	42.2	7.3	422
9-10	2.4	40.9	5.6	377
11-12	1.8	37.2	6.3	433
13-14	4.6	36.9	5.3	282
15-16	3.6	42.8	3.6	166

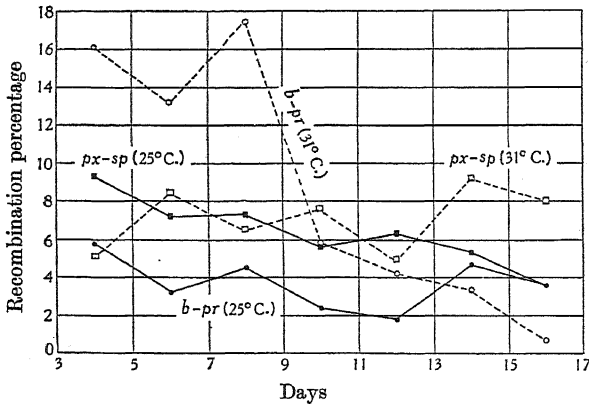


Fig. 4. Curves showing the percentage of recombination for the *b-pr* and the *px-sp* region in the second chromosome of *D. melanogaster* at 25° C. and 31° C. (exposed to this temperature at only larval and pupal stages). (Table IX.)

of the mother. Plough's (1921, p. 190) data also accord with my experimental result, at any rate in respect of the effect of high temperature.

In the autosomes of this species, Muller (1925) reports a typical case

of compensation. According to his data, a heavy dose of X-rays produced a decrease in crossing-over for the distal region, whereas it brought about an increase for the proximal region (near the spindle-fibre attachment). However, it has not been completely determined whether or not high temperature gives a result similar to that shown by X-ray irradiations¹. This has led me to perform the following experiment. The regions of the second chromosome of *D. melanogaster*, *black-purple*, *purple-plexus* and *plexus-speck* were selected. The method was the same as that employed in the preceding experiments. Table IX and Fig. 4 show the result of this experiment. As previous investigators have shown, the cross-over frequency of the *black-purple* region was increased markedly by high temperature, while no significant change worth mentioning was found in the *plexus-speck* region (Plough, 1917, 1921; Mavor and Svenson,

TABLE X.

Effect of X-ray irradiations on the cross-over frequency in the second chromosome of D. melanogaster.

Dosage: Coolidge tube with tungsten target, 50 kV, 4 ma, 21 cm., 30 min., non-filter.
Count was done by Muller's (1925) method.

Kinds	Regions			Total
	(<i>b-pr</i>)	(<i>pr-px</i>)	(<i>px-sp</i>)	
X-rayed	12.82 ± 0.97	41.57 ± 1.43	5.65 ± 0.69	1186
Control	2.31 ± 0.49	40.46 ± 1.58	6.08 ± 0.77	954
Diff./σ	10.51/1.09 = 9.6	1.11/2.14 = 0.5	0.43/1.04 = 0.4	

1924 *b*; Bergner, 1928; Kirssanov, 1931; Graubard, 1932). The result of X-ray irradiations also shows that the *px-sp* region is unaffected (Table X). Further, this region shows no compensatory change in the cross-over frequency according to the age of mother, as contrasted with the proximal region (*b-pr*).

However, more critical examination of the result in Table IX reveals that there is a considerable difference in the cross-over value of the *px-sp* region between the treated and non-treated series, in the first period (3rd-4th day). This suggests strongly that high temperature may bring about a decrease in crossing-over for the region distal to *px-sp*. In fact, Dobzhansky (1932 *a*) finds that the locus of this region (*px-sp*) seen under the microscope is not so distal as is suggested by the genetic map. To my regret we have no appropriate gene in the stocks in our laboratory for

¹ Very recently, Kirssanow (1933) finds that high temperature produces a decrease in crossing-over for the distal region (*roughoid-hairy*) in the third chromosome of *D. melanogaster*.

testing this point thoroughly. It is therefore highly desirable that an experiment on this point should be performed in some other laboratory.

(2) *Wavy variation of the cross-over frequency.*

The result shown in Figs. 1 and 2 implies another noteworthy phenomenon relating to non-inherited variation in crossing-over. In these figures, the cross-over frequency of the distal region, which has once been reduced to a minimum value by high temperature, exceeds the normal value at the period of its recovery, but then again decreases. A similar relation is also found in the region between *vermilion* and *singed* in Table III. In the proximal region also, an analogous phenomenon is frequently found in many experimental results, namely, the cross-over frequency which has once been increased by high temperature, is reduced to less than the normal value at the period of its recovery, but subsequently tends to increase (see Plough, 1917, Tables 13, 14, Figs. 7, 8; Stern, 1926; Bergner, 1928; the present author, Fig. 4 in this paper). However, it seems that no special attention has ever been paid to this phenomenon by the previous investigators of *D. melanogaster*. Such a fluctuation of the cross-over frequency is also seen in the variation in relation to age of females (Figs. 1-4; Bridges, 1927, 1929; Stern, 1926; Sturtevant, 1929). This special phenomenon is of great significance and apparently represents another basic property relating to non-inherited variation in crossing-over.

Thus far I have been concerned with deductions from the experimental results, but the question remains as to what kind of mechanism is responsible for such a change in crossing-over, and this I shall now attempt to discuss in the following section.

III. DISCUSSION OF THE RESULTS.

(1) *Regional differences in susceptibility.*

It has hitherto been assumed that the susceptibility to any stimulus is dependent on the distance from the spindle-fibre attachment (Plough, 1917, 1921; Mavor, 1924 *a*, *b*; Muller, 1925; Bridges, 1927, 1929; Graubard, 1932). If this assumption were adequate, the most distal region of the chromosome should show the least susceptibility. But, as is shown by many experimental results obtained in both *D. melanogaster* and *D. virilis*, this assumption can hardly hold true. Stimuli such as X-rays and high temperature cause a decrease in the crossing-over of the distal region, and an increase in that of the proximal region, whereas

no significant change is perceptible in the intermediate region (cf. section II). Of course there may be some exceptional cases. For example, the region between *plexus* and *speck*, or that between *yellow* and *cross-veinless*, which are located near the distal portion of the genetic maps of *D. melanogaster*, are little affected by treatment with X-rays or by high temperature. However, as pointed out before, these regions are relatively long in the absolute length of the chromosomes (Dobzhansky, 1932 *a, b*; Muller and Painter, 1932). It is therefore conjectured that the regions more distal to those given above are probably more sensitive to the stimuli. Nevertheless, as is shown by the data of Muller (1925) and those of the present author (*loc. cit.*), the region between *roughoid* and *hairy* (in the third chromosome of *D. melanogaster*) or that between *plexus* and *speck*, shows changes in crossing-over in two different directions (above and below the normal value), in accordance with the dosage of the stimuli. Such a phenomenon has already been suggested by Plough (1924) in connection with his radium experiment.

Thus it may be concluded that the susceptibility of one region to any stimulus is not constant, but varies according to the position of that region on the chromosome, and also to the dosage or the kind of the stimuli. This property should be taken into account in analysing the problems of the non-inherited variation in crossing-over.

(2) *The sensitive period for a stimulus.*

Plough (1917) concluded from his genetic and cytological data that the effect of temperature is restricted to the early oocyte stage. Bridges (Morgan *et al.* 1925), using Mavor's data of X-ray irradiations, has expressed the view that X-rays may produce the effect at any stage of oogenesis. Bergner (1928), however, considers from her data on the prolongation of larval and pupal stages and on enforced sterility of adult females, that the effect is restricted to the middle oogonial stage. Thus, at present, opinions are varied in regard to this question. Nevertheless, as I have already stated, the fact that the frequency of crossing-over shows a wavy fluctuation, so to speak, strongly supports Bridges' assumption, according to which the stimulus produces its effect at any stage of oogenesis, because it is very improbable that the effect upon only one stage of oogenesis should result in such a periodic change in the cross-over frequency.

In my opinion the following assumption seems to be the most adequate, viz., that all the oogonia and oocytes have a uniform tendency to give a certain cross-over value for one region at a given period; in other words,

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the *a priori* frequency of crossing-over in one region of the chromosome is the same at a given period of development in every oogonium and in every oocyte, and the frequency is affected equally in all cells by a certain stimulus.

For example, let us assume the case where the *a priori* frequency of crossing-over in every ovarian cell has a tendency to assume a greater value in the proximal portion and a smaller value in the distal portion, than the normal value, through the influence of a certain stimulus. In this case, if the stimulus is removed, the cross-over frequency of the proximal portion of the chromosomes in every ovarian cell may begin to decrease at once or after a short time, while that of the distal portion may increase contrarily; stated in another way, the *a priori* frequency of crossing-over in every ovarian cell may return to a state of equilibrium with lapse of time. However, during this period, the cells will continue to divide and grow, some oocytes may be produced, and some oogonia may pass the stage of crossing-over. Thus, the whole system is to be thought of on kinematical lines.

(3) *Elastic movement of the cross-over frequency on the chromosome.*

To obtain a clearer view of the matter we may consider the case of a "spring-balance." On suspending a weight of m gm. the spring would at once expand and stay at a certain point where the elastic force and gravity are in a state of equilibrium. If, after this motion has come to a full stop, we draw the spring further down, x from the above point, and then let it go, it would lead to a movement which may be expressed by the following formula:

$$m \frac{d^2x}{dt^2} = -a^2x + mg \quad \dots\dots(1).$$

(Here a = elastic coefficient, m = mass, $\frac{d^2x}{dt^2}$ = acceleration, x = co-ordinate of an oscillatory particle, g = gravity.)

If we put $n^2 = \frac{a^2}{m}$, the above formula is transformed:

$$x - \frac{g}{n^2} = A \cos nt + B \sin nt \quad \dots\dots(2).$$

(A and B are constants.)

This formula (2) indicates the "simple harmonic motion" which oscillates up and down with the position $x = \frac{g}{n^2}$ as the centre. This position

is of course identical with that at which the spring stays when a weight of m gm. is suspended on it.

It is, however, impossible to find such a simple harmonic motion in nature. All vibration decreases its amplitude with lapse of time, and at last comes to a stop in a state of equilibrium. This is due to the so-called "resistance." In the formula illustrating such a relation, $m \frac{d^2x}{dt^2} = -a^2x - k \frac{dx}{dt}$, we can distinguish the following three types of motion, in accordance with the strength of resistance (k = coefficient of resistance, $\frac{dx}{dt}$ = velocity). In the first the amplitude decreases gradually and it reaches the state of equilibrium without any oscillation; in the second, the amplitude exceeds once the state of equilibrium and then returns to it without any further oscillation; and in the third, the amplitude shows the motion called "damped oscillation" (Fig. 5).

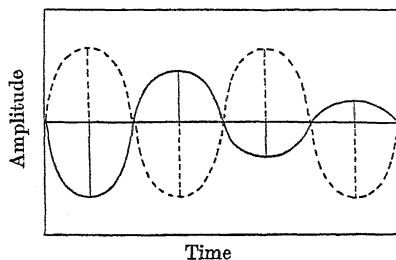


Fig. 5. Curve illustrating "damped oscillation." Curve given by the dotted line shows "simple harmonic motion."

This last type of motion is of especial interest and importance since it is closely analogous to that found in many experimental results shown in section II. This remarkable resemblance apparently offers an explanation of the second basic property stated before, and also supports the view that *the non-inherited variation in crossing-over is caused by an elastic movement of the cross-over frequency which oscillates from the distal to the proximal direction of the chromosome and vice versa*. However, it seems premature to conjecture whether or not this phenomenon is due to elasticity of the chromosome itself (especially of chromonemata), or due to the shifting movement of the genes on the chromosome, or to some change in density and viscosity of the chromosomal substance (especially of matrix).

(4) *The velocity of movement of the cross-over frequency.*

The validity of the above assumption may be also supported by the following experimental facts. As stated before, the mode of movement

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of the cross-over frequency on the chromosome seems to be comparable with the motion of a spring-balance. Therefore the response of the cross-over frequency to a certain dose of any kind of stimulus (X-rays or high temperature) will correspond with the response of the spring-balance to a certain weight value. As an example, let us take the case of high temperature. However, we have to consider here the following two possible cases separately: viz. (a) the case where the interval of treatment is constant, but temperature is different, and (b) the case where temperature is constant, but the interval of treatment is different.

(a) *The case where the interval of treatment is constant, but temperature is different.*

For the experiment the *black-purple* region of the second chromosome of *D. melanogaster* was used. Three groups of adult females of the constitution $\frac{b, pr, (c), (px), (sp)}{+, +, (+), (+), (+)}$, which had emerged within a day at 25° C., were mated to $C_{\Pi L}, b, pr$ males and bred for 14 days at 25° C. (as control), 29° C. and 31° C. respectively. In the course of the experiment, each female was transferred to a fresh vial almost every second day after being kept during the 1st–2nd day in a mass culture. The result is given in Table XI and Fig. 6.

Looking at the curves in Fig. 6 closely, we notice that the cross-over value does not change until a period of several days has passed after the first treatment, whereupon it begins to vary and reaches the maximum point which is roughly proportional to the index of temperature applied. Such a relation will be expected also in the case of the spring-balance, and is known as "Hooke's law."

TABLE XI.

Variation in crossing-over in the black-purple region of the second chromosome of D. melanogaster at different temperatures.

$\frac{b, pr, (c), (px), (sp)}{+, +, (+), (+), (+)} \times C_{\Pi L}, b, pr.$						
Days after emergence	Control (Emerged and bred at 25° C.)		Series I (Emerged at 25° C., bred at 29° C.)		Series II (Emerged at 25° C., bred at 31° C.)	
	R.V.	Total	R.V.	Total	R.V.	Total
3–4	5.7	525	5.8	1387	6.2	1355
5–6	3.2	502	6.8	1371	14.8	1427
7–8	4.5	422	8.6	1119	16.5	1336
9–10	2.4	377	9.2	879	15.7	1177
11–14	2.9	715	8.3	1260	14.7	1813

The control value was taken from the result of Table IX.

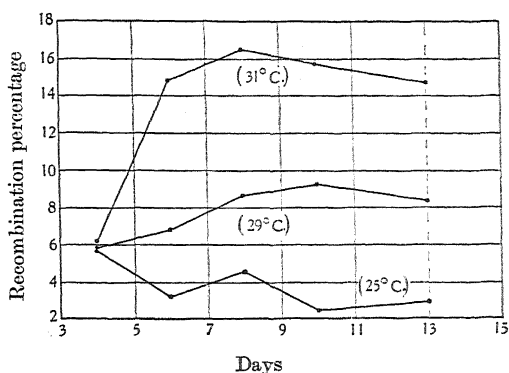


Fig. 6. Curves showing the variation in crossing-over for the *b-pr* region in the second chromosome of *D. melanogaster* at different temperatures. (Table XI.)

It is, however, to be noticed that the difference in temperature cannot be compared directly with that in the weight value. For as Plough 1917, pp. 160-1) has already pointed out in his classical study, the maximum fluctuation value of crossing-over is not directly proportional to the index of temperature.

(b) *The case where temperature is constant, but the interval of treatment is different.*

For this purpose, only the case of 30° C. was selected as an example. Five groups of females of the constitution $\frac{b, pr, (c), (px), (sp)}{+, +, (+), (+), (+)}$, which had emerged at 25° C. within a day, were exposed to 30° C. for 1, 2, 3 and 4 days respectively, while the remaining one group was set aside as control without applying any treatment. The females of these groups were mated to the C_{HL}, b, pr males and bred at 25° C. after the treatment. Each female was transferred to a fresh vial almost every day. Table XII shows the results of this experiment.

In this table, it is shown that: (i) the effect of high temperature (30° C.) appears on about the sixth day after the first treatment; (ii) 1 or 2 days' exposure gives rise to a slight variation in crossing-over¹ (Series I

¹ According to the result obtained by Mavor and Svenson (1924 b), the cross-over value for the black-purple region is markedly influenced by 2 days' exposure to the same temperature (30° C.). But, in my experiment such a great variation was not observed. The difference found between these two experiments, may partly be due to the difference of the stocks used. For the *b, pr, c, px, sp* stock in our laboratory always gives a lower cross-over value than the standard one. This is probably due to the speciality of the stock used, as in Bergner's (1928, pp. 111-12) experiment.

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and II); (iii) but, 3 or 4 days' exposure seems to yield the maximum fluctuation value of crossing-over for the given temperature (Series III and IV); (iv) the effect lasts approximately as long as the period of treatment, but is slightly prolonged.

Thus, from the results obtained in the two cases (a) and (b), and also from those shown in section II, we may conclude that the velocity of the movement of the cross-over frequency on the chromosome is rather slow.

TABLE XII.

Variation in crossing-over in the black-purple region of the second chromosome of D. melanogaster at 30° C.

$\frac{b, pr, (c), (px), (sp)}{+, +, (+), (+), (+)} \times C_{\text{II}}, b, pr.$										
Days after emergence	Control (Emerged and bred at 25° C.)		Series I (Emerged and bred at 25° C.; 1st day, exposed to 30° C.)		Series II (Emerged and bred at 25° C.; 1st-2nd day, exposed to 30° C.)		Series III (Emerged and bred at 25° C.; 1st-3rd day, exposed to 30° C.)		Series IV (Emerged and bred at 25° C.; 1st-4th day, exposed to 30° C.)	
	r.v.	Total	r.v.	Total	r.v.	Total	r.v.	Total	r.v.	Total
3-4	6.2	308	7.2	663	5.3	435	6.4	639	5.4	636
5-6	4.9	329	4.1	753	3.6	247	4.9	526	2.5	283
7	2.7	339	5.8	431	5.4	373	10.8	287	7.2	431
8	2.4	379	5.8	326	7.0	271	14.7	218	9.5	374
9	4.6	281	5.4	430	6.0	350	9.9	282	10.0	449
10	2.1	335	2.1	425	3.9	335	12.1	281	11.7	401
11	2.3	344	—	—	4.1	270	8.1	261	10.8	409
12	4.9	306	—	—	—	—	5.8	291	8.2	406
13	5.7	230	—	—	—	—	—	—	5.5	362
14	6.6	183	—	—	—	—	—	—	4.8	333

This is probably due to "resistance" which hinders such movement of the cross-over frequency, and it may have also a close bearing on the phenomenon of "damped-oscillation" in the cross-over frequency. A clearer knowledge of this point may throw light on the new interpretation of "the incubation period" which has been studied by Plough (1917) and Bergner (1928).

(5) *The problem of the variation in crossing-over in relation to age of female.*

The elasticity hypothesis seems to find a good application in the solution of this problem. In the middle portion of the second and third chromosomes of *D. melanogaster*, Plough (1917, 1921) and Bridges (1927, 1929) found that the cross-over value is highest in the beginning, and decreases rather rapidly to the minimum at about the tenth day, then a second rise reaches a level at about the nineteenth day, whereupon a

second fall and third rise follow. Thus, according to the results of their experiments, the curve has roughly a W-form.

In the *X*-chromosome of *D. melanogaster*, Stern (1926) reports a slight decrease in crossing-over in the *Bar-bobbed* region (near the spindle-fibre attachment) at about the fourth day. On the contrary, Plough (1921) found no significant change in crossing-over in the regions from *scute* to *forked*. However, according to my experimental results obtained in both *D. virilis* and *D. melanogaster*, there is little doubt that there is a noticeable change of crossing-over with the age of the female in the distal region except *px-sp*; the frequency curve here has roughly an M-form. Sturtevant (1929) has obtained a similar result in the distal region of the sex-chromosome of *D. simulans*.

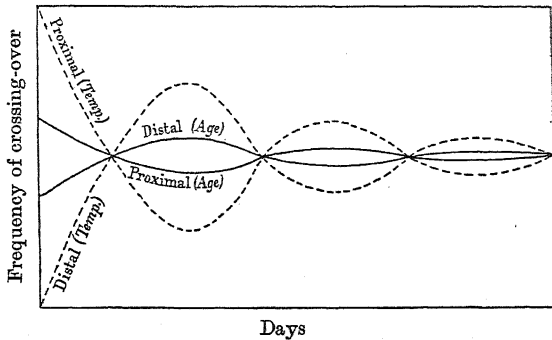


Fig. 7. Schematic curves showing the wavy variation in crossing-over in relation to the age of the females (25° C.) and to the heat treatment.

To meet this question, it does not seem absurd to assume that the *a priori* frequency of crossing-over is moved toward the proximal direction by some internal causes in the larval or pupal stage. If so, as stated in the preceding sections, the cross-over frequency which has started from a certain initial value may fluctuate around a definite value with age of the females, and finally reach a state of equilibrium (Fig. 7). Thus, in my opinion, the age variation as well as the induced variation is an inevitable result caused by the initial vibration of the cross-over frequency. Of course, for the validity of this assumption, it is necessary to postulate that no internal cause which produces any variation in crossing-over is existent throughout the adult stage of the female. This point is left for future study, as well as the problem concerning the correlation between the productivity of the female and the age variation in crossing-over (Morgan, Bridges, Schultz, 1931).

(6) *The direct causes of variation in crossing-over.*

Bridges (1915, 1927, 1929) tried to explain the variation in crossing-over by these two inferences, viz. (a) the change in the coefficient of crossing-over, and (b) the change in the internode length. But these cannot completely explain all the phenomena relating to crossing-over, as he himself admitted (Bridges and Morgan, 1919, pp. 188-9). Recently, Dobzhansky (1931) advanced an assumption that has its ground in the failure of synapsis. This is analogous to the conception advanced independently by the present author (1931, 1932 *a*, and in the press), which is based on the phenomenon of incomplete synapsis. However, the question remains as to what kind of interrelationship exists among the following phenomena: change in the coefficient of crossing-over, change in the strength of interference, and change in the mode of synapsis. This problem will be taken up in another paper. For the present it may be disregarded, since the region taken in each experiment is rather short, and the influence of double cross-overs and that of incomplete synapsis may be almost neglected.

IV. SUMMARY AND CONCLUSION.

1. The causes of variation in crossing-over may be classified roughly into two categories, viz. (a) inherited or permanent causes, and (b) non-inherited or temporary causes. This paper deals with the latter. Many experimental results obtained both in *D. virilis* and *D. melanogaster* show that: (1) if the cross-over frequency decreases in the distal region, it tends to increase in the proximal region, and *vice versa* (compensatory relation of the cross-over frequency); (2) generally speaking, the after-effect of any stimulus on crossing-over appears in the form of a wavy curve (Fig. 7).

These two basic properties lead to the conclusion that the stimuli affect all the stages, instead of a single stage, of oogenesis.

2. From the properties mentioned above, it seems well to compare the mode of non-inherited variation in crossing-over with the elastic movement of a spring-balance. Thus, the following hypothesis has been deduced: *the non-inherited variation in crossing-over has its cause in the elastic movement of the a priori frequency of crossing-over, which oscillates from the distal to the proximal direction of the chromosome, and vice versa.*

3. Based on this assumption, the curve of variation of crossing-over in relation to age of female was explained.

I wish to acknowledge my indebtedness to Prof. Dr Taku Komai for his invaluable advice and criticism, and for his taking trouble in looking through the original manuscript. To Dr Mitsushige Chino also, I am indebted very much for his valuable advice and kindness, especially on the side of materials. Further, I wish to express my gratitude to Prof. Dr U. Yoshida and the staff of the Physical Laboratory of Kyoto Imperial University, for their kindness in granting me the use of the X-ray apparatus. My sincere thanks are also due to Prof. Dr R. C. Punnett, who showed me much favour in publishing this paper.

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THE DISTRIBUTION OF SEX-FACTORS IN THE X-CHROMOSOME OF *DROSOPHILA* *MELANOGASTER*.

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(With Sixteen Text-figures.)

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INTRODUCTION.

AN important step in the development of the theory of sex determination was made by Bridges (1921, 1922, 1923, 1925, 1932) who discovered triploids, intersexes and supersexes in *Drosophila melanogaster*. In these types the change in sex depends not only on the number of X-chromosomes but also on the number of sets of autosomes. The primitive notion according to which sex depends merely upon the presence of one versus two X-chromosomes became clearly inadequate, and had to be replaced by a conception more in accord with facts. Bridges suggested that sex is influenced by numerous genes located in different chromosomes (perhaps even by all genes present in the germ plasm). Some of the genes modify development towards femaleness and others toward maleness. The sex of an individual depends, then, upon the degree of preponderance of female-determining genes over male-determining ones, or *vice versa*. This view is known as the theory of genic balance. It implies

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that sex is a quantitatively variable character, a concept arrived at by Goldschmidt (1920, 1927, 1931), and elaborated by the latter into a generalised theory.

The sex-determining rôle of the *X*-chromosome of *Drosophila* and of other forms possessing the *X-Y* type of sex determination is due, according to Bridges, to a preponderance of female modifiers over male modifiers in this chromosome. The influence of autosomes on sex was quite consistently interpreted by Bridges as indicating that in the autosomes male-determining genes are either more numerous or stronger than female-determining ones.

This interpretation agrees with the facts discovered by Bridges and by later workers, but it is not the only possible interpretation. It is equally plausible to suppose that there is a single female-determining gene in the *X*-chromosome (the female sex differentiator), and that the rest of the genes located in the *X* are not concerned with sex at all.

Likewise, the sex-determining rôle of the autosomes may depend upon the presence of a single male-determining gene (the male sex differentiator) located in one of them, the majority of genes being sexually neutral. One may, perhaps, account for the sex-determining function of the autosomes even without assuming any autosomal sex genes. Sex may be determined by the ratio between the number of *X*-chromosomes present in the cell and the size of that cell. Cell size in most organisms (including *Drosophila*) is positively correlated with the volume of chromosome material contained in the nucleus. Two *X*-chromosomes together with two sets of autosomes produce a diploid female. The same two *X*-chromosomes together with three sets of autosomes give, however, a triploid intersex, having larger cells than those found in diploid females (Dobzhansky, 1929). Two *X*'s with four sets of autosomes give, presumably, a tetraploid male with still larger cells. The influence of the autosomes on sex may be, therefore, indirect, and due to the increase of the cell size produced in polyploids. In organisms in which polyploidy results in little or no increase of cell size (see the experiments of Wettstein, 1924, 1928) an alteration of the number of autosomes need not affect sex. Perhaps the discrepancy between the sex-determining mechanisms found in *Drosophila* and in the honey-bee (haploid *Drosophila* is a female, haploid bee is a male) may be accounted for by some such relationship.

The problem of the distribution of sex-determining factors in the chromosomes may now be studied experimentally. Muller's discovery of the production of chromosomal aberrations by X-ray treatment

furnishes the method. Chromosomes can be fragmented by X-rays, and the length of the resulting fragments can be determined both genetically and cytologically. Fragments of known length can be added to the normal chromosomal complements of females, males or intersexes, and the effect of the addition on the expression of sexual characters studied. This method is, of course, applicable to the analysis of the distribution of sex-factors in the *X*-chromosome as well as in the autosomes.

The present paper is devoted to the study of the topography of the *X*-chromosome with respect to the localisation of the sex factors. The work was done in the years 1928-33 at the California Institute of Technology, Pasadena. The results were reported in a preliminary form by Dobzhansky and Schultz (1931). The authors wish to express their sincerest appreciation to Drs T. H. Morgan, C. B. Bridges and A. H. Sturtevant for their advice and criticism. We are also much indebted to Profs. H. J. Muller and C. P. Oliver and to Mr J. Bonner and Mrs L. V. Morgan for the use of their material and also of unpublished data pertaining thereto.

PLAN OF THE EXPERIMENTS.

On Bridges' assumption that female modifiers predominate in every section of the *X*-chromosome, the addition or a subtraction of any fragment of the *X* to or from the chromosomal complement of a male, a female, or an intersex should produce a part of the effect produced on these forms by additions or subtractions of whole *X*-chromosomes. On the other hand, if there were a single sex differentiator, or a group of strong female modifiers concentrated in a relatively short section of the *X*-chromosome, then the addition or subtraction of the section containing the sex differentiator should produce the effect of the addition or subtraction of whole *X*-chromosomes, at least as far as the sexual characteristics are concerned. The addition or subtraction of sections other than that carrying the sex differentiator should, presumably, have no effect on the sexual characters.

The alternatives formulated above can be put to a test by studying females, males or intersexes carrying duplications or deficiencies for various sections of the *X*-chromosome. If a section of the *X* contains some, but not all, of the female modifiers present in the whole *X*, then a female having two complete *X*'s plus a duplication for this section should have some (but not all) of the characteristics of a superfemale; an intersex having two complete *X*'s plus the duplication should be more female-like than the intersex free from the duplication; a male

having one complete *X* plus the duplication should show signs of beginning intersexuality. Deficiencies should produce effects opposite to those produced by duplications.

Intersexes are more critical for such studies than females or males. This is due to the fact that intersexes carrying duplications or deficiencies are less frequently inviable than males and females with the same duplications and deficiencies. Moreover very small alterations of the sexual balance produce easily noticeable changes in the morphology of intersexes but not in females and males. Thus some modifying genes and environmental agents may influence the sexual type of intersexes but not of females and males (Dobzhansky, 1930*a*, *b*).

The technique of obtaining individuals carrying duplications and deficiencies will be described below. In every case the experiments are so arranged that individuals carrying duplications and those free of them, but otherwise of identical genetic constitution, are obtained in the same mating and develop side by side in the same culture bottle. This insures the highest possible degree of uniformity of environmental conditions, to which intersexes are known to be especially sensitive. Most experiments were carried out at room temperature (20°–22°) and not in an incubator, since some weak types of flies do not survive at higher temperatures. Overpopulation of culture bottles was avoided by allowing only one triploid female to oviposit in each bottle.

Intersexes vary greatly in their sexual characteristics. Some closely resemble normal males and others resemble normal females. Still others have various proportions of female and male characters. To make the study of the influence of duplications on intersexes quantitative, the latter are divided into six arbitrary classes. The classification is, for convenience sake, based on external sexual characteristics only, but, as shown by Dobzhansky and Bridges (1928) and Dobzhansky (1930), there is in intersexes a rather high correlation between the external characters and the structure of the internal reproductive organs. The six classes may be defined as follows:

Class I. Extreme male type intersexes. Genitalia and coloration of the abdomen male. Penis and genital arch symmetrical. Sex combs present. Anal tubercle of male or female type.

Class II. As above, but penis and genital arch asymmetrical.

Class III. Intermediate intersexes. Neither male nor female external genitalia present, or genitalia extremely rudimentary. Anal tubercle female. Coloration of the abdomen male. Sex combs present.

Class IV. As above, but female genitalia present. Vaginal plates asymmetrical.

Class V. Female type intersexes. Female genitalia present. Vaginal plates symmetrical. Coloration of the abdomen female (rarely intermediate). Sex combs present at least on one leg.

Class VI. Extreme female type intersexes. As above, but sex combs absent on both legs and coloration of the abdomen female.

Intersexes carrying duplications are compared with those free of them by determining the frequencies of the individuals belonging to the different classes. It is evident that the presence of duplications carrying female modifiers should produce a shift to the female direction in the average type of intersexes.

THE INERT REGION.

The *Y*-chromosome of *D. melanogaster* is longer and more voluminous than the *X* of the same species. Nevertheless, the small number of genes located in the *Y* contrasts sharply with the very large number known in the *X*. According to Stern (1926, 1927) the *Y* carries only one gene producing a visible effect, namely bobbed, and two "fertility factors" the absence of which causes sterility of the XO males. Because of the scarcity of known genes the material composing the *Y*-chromosome is considered genetically inert. Recently it was found (Painter, 1931; Muller and Painter, 1932; Dobzhansky, 1932*a*) that nearly all the sex-linked genes of *Drosophila* are located in the distal two-thirds of the *X*-chromosome (the black part of the chromosome, Fig. 1). The proximal one-third contains a single known gene (bobbed), and must be considered inert in the same sense as the *Y* (the stippled part in Fig. 1). It is very probable that the inert region of the *X* is homologous to a section of the *Y*-chromosome.

It is important to know whether the "inert" material in the *X* and the *Y* carries any factors modifying the sex balance. Triploid females crossed to normal males produce two kinds of intersexes, some having two maternal *X*'s and a paternal *Y*, and others having one maternal *X*, one paternal *X*, and no *Y*. The two kinds of intersexes can easily be distinguished if a triploid female homozygous for a sex-linked recessive is crossed to a male carrying the dominant allelomorph. In our experiment 3*N* females homozygous for yellow were crossed to wild-type (Oregon) males. Some intersexes were yellow (*XXY*) and others wild-type (*XX*). Their comparison is shown in Table I (*n* in this and in the following tables indicates the number of intersexes studied).

There is no significant difference between the XX and XXY intersexes. Unless the influence of the Y-chromosome in this experiment was exactly counterbalanced by the modifiers introduced by the "Oregon" stock, it is legitimate to conclude that the Y as a whole has no influence on the sexual balance.

TABLE I.

Type of intersexes in the cross 3N yellow ♀ × wild-type ♂.

	I	II	III	IV	V	VI	n	Mean type
Yellow	15	44	24	17	—	—	100	2.41 ± 0.09
Wild-type	35	111	64	48	5	—	263	2.53 ± 0.06

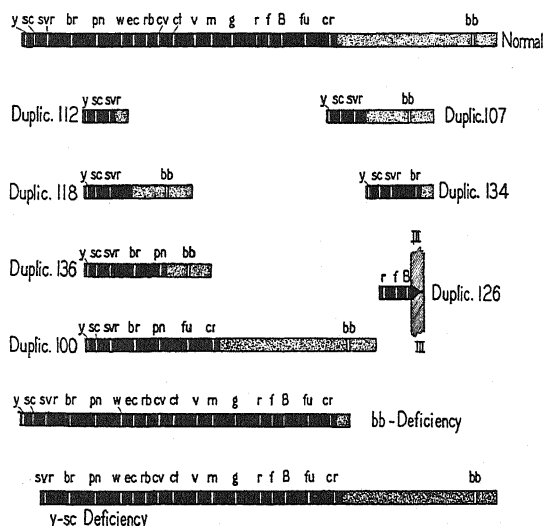


Fig. 1. Scheme of the structure of the normal X-chromosome of *Drosophila melanogaster*, and of some of the duplications and deficiencies. The inert region is stippled; the active portion is represented in black. Letters indicate the location of some of the genes mentioned in the text. B, bar; bb, bobbed; br, broad; cr, carnation; ct, cut; cv, crossveinless; ec, echinus; f, forked; fu, fused; g, garnet; m, miniature; pn, prune; r, rudimentary; rb, ruby; sc, scute; svr, silvery; v, vermillion; w, white; y, yellow. The locus of lozenge (lz), not shown in the diagrams, lies between ct and v.

To test for the possible influence of the inert region of the X-chromosome bobbed deficiency (Sivertzev-Dobzhansky and Dobzhansky, 1933) was used. Bobbed deficiency (Fig. 1) represents a loss of about one-third of the length of the X-chromosome, which is probably the entire inert region, or at least a very large part of it. Triploid females homozygous for yellow were crossed to bobbed-deficiency males whose X-chromosome carried the wild-type allelomorph of yellow. The results are shown in Table II.

Obviously no influence is produced by the deficiency. It follows that either the inert region of the X -chromosome is "inert" also in respect to the sex balance, or the section of the chromosome lost in bobbed deficiency has equally strong female and male modifiers. In view of what we know about the inert region, the former conclusion seems more probable than the latter.

TABLE II.

Type of intersexes in the cross 3N yellow ♀ × bobbed deficiency ♂.

	I	II	III	IV	V	VI	n	Mean type
Yellow	10	47	26	23	1	—	107	2.62 ± 0.07
Wild-type (<i>bb^{def}</i>)	30	79	48	50	4	—	211	2.61 ± 0.09

DUPLICATIONS FOR THE LEFT END OF THE X -CHROMOSOME.

Duplications for sections of the X -chromosome may be obtained by irradiating normal *Drosophila* males with X-rays and crossing them to females having attached X -chromosomes (\widehat{XX}) homozygous for a desired set of sex-linked recessives (Painter and Muller, 1929). In a part of irradiated spermatozoa the X is fragmented, some fragments are frequently lost, and such spermatozoa fertilising the \widehat{XX} eggs produce females possessing a fragment (duplication) of an X -chromosome besides the two complete attached X 's. If the X of the treated male contains dominant allelomorphs of the genes present in the \widehat{XX} females, the duplication carrying daughters can be recognised by their phenotype. The most frequent type of duplications obtained by this method is a short chromosome consisting of two parts, one of which is homologous to the left (distal) end of the X , and the other to the right (proximal) end. The proximal end of the X is the inert region, but it includes the spindle fibre attachment, and consequently the duplication chromosomes have their own spindle fibres and can therefore be regularly transferred from one cell generation to the other. The origin of these duplicating fragments is evidently due to a loss of the middle portion of the normal X -chromosome followed by a reunion of the end parts. If the \widehat{XX} duplication carrying females are crossed to normal males, a part of their sons receives a complete X -chromosome (from the father) and a fragment (duplication) of another X from the mother. If the duplication involved is sufficiently short, duplication carrying males survive and are fertile.

For the purposes of the present work a series of duplications for the left end of the X -chromosome was studied. The methods used for determining the loci contained in these duplicating fragments, as well as

their cytology, are described in Dobzhansky (1932a), and Sivertzev-Dobzhansky and Dobzhansky (1933). The various duplications are schematically represented in Fig. 1. It may be noted here that duplications which genetically seem to be identical may be cytologically of different lengths. For instance, duplications 112, 107 and 118 all carry the loci, *y*, *sc* and *svr* (Fig. 1), and the latter two also carry *bb*. Duplication 112 is cytologically very short, 107 is much longer, and 118 is still longer (Dobzhansky, 1932). Duplication 134 contains *y*, *sc*, *svr*, *kz*, and *br*, but it is cytologically shorter than either duplications 107 or 118. These apparent discrepancies are due, first, to the varying amounts of the inert region included in the duplications, and, second, to the fact that between the genes *svr* and *br* there is a cytologically rather long distance in which no genes are known, and varying amounts of which may be included in the duplications (the locus of the gene *kurz*, not represented in Fig. 1 is located cytologically at *br*).

Diploid females and males carrying duplications 112, 107, 118, 134 and 136 are fertile and nearly normal in appearance, except duplication 136 males which are smaller, have broader wings, and shorter bristles than normal males. Fertility of the duplication carrying individuals, except that of the duplication 136 males, is not much below normal. The only special characteristics produced by these duplications are the appearance of bristles on the occiput, and sometimes (mainly in males) of a few short bristle-like hairs along the second and third longitudinal veins on the wing. The occipital bristles are rather irregular, asymmetry is very frequent. Sometimes one side of the head has two such bristles and the other side has none, and sometimes they are altogether wanting. Both the occipital bristles and the bristles on the wing-veins are characters produced regularly by the sex-linked dominant gene Hairy-wing whose locus is inseparable from that of *y* and *sc* (Fig. 1). Hairy-wing males and even heterozygous Hairy-wing females have, however, all these characters much more strongly pronounced than the duplication carrying individuals not possessing the Hairy-wing gene. Hairy-wing gene plus the duplications shows a strong exaggeration of the Hairy-wing characteristics. Likewise, duplications produce an exaggeration of the effect of the third-chromosome recessive hairy, making it semi-dominant. Hairy, whose locus has clearly nothing to do with the region of the X-chromosome covered by the duplications, causes the appearance of bristles on the longitudinal veins on the wing, and is, therefore, similar in its effect to the duplications. In spite of the considerable interest of all these characteristics of the duplications from the developmental standpoint,

we need not discuss them here in more detail since they do not affect sexual characters.

Males were obtained which had a complete X-chromosome carrying yellow, and which carried duplications 112, 107, 118, 134 or 136. Since all these duplications cover the locus of yellow, and since they contain the wild-type allelomorph of this gene¹, duplication males are wild-type in appearance. When such males are crossed to triploid females homozygous for yellow, two kinds of intersexes are produced in the offspring: yellow and wild-type. The former are free of the duplications, the latter carry them. A comparison of these two kinds of intersexes as to the sexual type is shown in Tables III and IV.

TABLE III.

Type of intersexes in the cross 3N yellow ♀ × yellow/duplication ♂.

Dupli- cation	non-yellow							yellow (control)					
	I	II	III	IV	V	VI	n	I	II	III	IV	V	n
112	—	6	10	100	44	1	161	40	50	42	53	1	186
107	—	—	5	129	85	9	228	58	116	74	58	—	306
118	—	—	10	91	87	15	203	66	59	28	16	—	169
134	—	—	2	36	47	11	96	61	188	91	91	2	433
136	—	—	1	12	32	14	59	130	42	18	17	—	207

TABLE IV.

Mean type of intersexes carrying the duplication and free of it.

	Duplication	Control
112	4.15 ± 0.055	2.59 ± 0.08
107	4.43 ± 0.04	2.43 ± 0.06
118	4.53 ± 0.05	1.97 ± 0.075
134	4.70 ± 0.07	2.50 ± 0.06
136	5.00 ± 0.07	1.62 ± 0.07

Tables III and IV leave no doubt that the presence of the duplications shift the type of intersexes toward femaleness. The shift is statistically significant in every case. Among the intersexes carrying duplications individuals of the type VI are frequently found (Table III). They are very extreme female type intersexes, having no obvious male characteristics; they are practically never found in the absence of duplications. Dissection of such intersexes showed that they have well developed female genital ducts, but their ovaries were found still underdeveloped, so that such intersexes clearly cannot be fertile.

Different duplications modify the type of intersexes to a different extent. Duplication 112 produces least, and 136 produces the greatest effect. The correspondence between the cytological length of a duplication

¹ Duplication 136 contains yellow-2 in the fragment.

and the magnitude of its effect on the intersexes is, however, incomplete (cf. Tables III, IV, and Fig. 1). Duplication 134 produces more effect than duplication 107, although the former is cytologically shorter than the latter. This apparent discrepancy is due to the fact that the cytological length of the duplications is to a large degree dependent upon the amount of the inert region they include (see above). If, however, one takes into account only the sections of the left end of the *X*-chromosome included in the duplications (Fig. 1) the correspondence between their lengths and the greatness of the effect on intersexes is perfect. This corroborates the conclusion reached above that the inert region has no influence on the sexual balance, and shows that the material located in the left (distal) end of the *X*-chromosome is female-modifying. This is true for the *y-sc-svr* region, for the *svr-br* region (since duplication 134 produces more effect than 112, 107 and 118), and for the *br-pn* region (since duplication 136 produces more effect than 134) (cf. Fig. 1 and Tables III, IV).

y-sc DEFICIENCY.

Sturtevant and L. V. Morgan (unpublished) discovered mutants which behave as if their appearance were due to deficiencies involving the loci of yellow and scute (Fig. 1). The *y-sc* deficiency produces an exaggeration of the effects of the included genes (*y* and *sc*), and is lethal in the male. The deficiency was not studied cytologically, but it is very unlikely that such a study would give any decisive result since the region lost in the deficiency must be extremely short in cytological terms. Duplication 112 which contains the loci *y*, *sc* and *svr*, and also a section of the inert region is no longer than one diameter of the fourth chromosome (see Dobzhansky, 1932 *a*); the region lost in *y-sc* deficiency must, evidently, be still shorter.

If the *y-sc* section of the chromosome contains genes modifying the sexual balance toward femaleness, the presence of the *y-sc* deficiency in the intersexes should produce a shift in their type toward maleness. To test this expectation it was necessary to obtain *y-sc* deficiency males. Such males, as mentioned above, are inviable. The difficulty was obviated by the following method. Females carrying one *X*-chromosome with the *y-sc* deficiency and the dominant Bar, and another *X* with the recessive yellow, and the dominant Hairy-wing (which is never separated by crossing over from *y-sc* deficiency) were crossed to males carrying yellow and duplication 107 (which covers the *y*, *sc* and *svr* loci, Fig. 1). In the offspring non-yellow, Bar, non-Hairy-wing males were obtained. Such

males carry the *y-sc* deficiency Bar chromosome and the duplication 107. The lethal effect of the deficiency is suppressed by the duplication. Such males were crossed to triploid females homozygous for yellow. Four kinds of intersexes appeared in the offspring. They are: (1) yellow non-Bar, which carry neither the deficiency nor the duplication, (2) yellow Bar, carrying the deficiency but not the duplication, (3) non-yellow Bar, carrying both the deficiency and the duplication, and (4) non-yellow non-Bar which carry the duplication but not the deficiency. These intersexes were classified as to their sexual type. The results are shown in Table V.

TABLE V.

Type of intersexes in the cross 3N yellow ♀ × y-sc deficiency Bar/Dup. 107 ♂.

	I	II	III	IV	V	VI	n	Mean type
Yellow	11	24	14	17	—	—	66	2.65 ± 0.13
Yellow-Bar	97	9	—	—	—	—	106	1.085 ± 0.09
Bar	12	42	40	50	—	—	144	2.89 ± 0.08
Wild-type	—	1	2	15	26	6	50	4.68 ± 0.11

The data presented in Table V prove that: (1) the deficiency shifts the type of intersexes toward maleness, (2) the duplication produces a shift toward femaleness, (3) simultaneous presence of the deficiency and the duplication produces a shift toward femaleness, although not as strong a one as that produced by the duplication alone. The last point (3) requires a comment. Individuals carrying the deficiency and the duplication have a very small net duplication for the locus *svr* (since the duplication covers that locus and the deficiency does not, Fig. 1). The fact that this very small duplication seems to produce a slight shift toward femaleness proves that the section of the chromosome containing *svr* carries female modifiers.

DUPLICATIONS FOR THE RIGHT END OF THE X-CHROMOSOME.

As shown above, the right one-third of the X-chromosome is composed of inert material and contains no sexual modifiers. Some duplications are known that cover the right end of the genetically active part of the X-chromosome (located approximately at the middle of the cytologically visible chromosome, Fig. 1). The effect of these duplications on sex is to be described here.

Mrs L. V. Morgan (T. H. Morgan, Sturtevant and Bridges, 1928) has studied a duplication which includes the *y-pn* section of the left end, and the *fu-bb* section of the right end of the chromosome (Fig. 1). This

duplication is designated as the duplication 100 (referred to in Dobzhansky and Schultz, 1931, as the duplication L. V. M.). It arose spontaneously, without X-ray treatment, but the method of its origin is presumably the same as that of the duplications described above: the *w-B* section of the *X* was lost, and the *y-pn* and *fu-bb* sections fused together to form the "new" chromosome that was added to the normal chromosome complement of females and males as a duplicating fragment. The duplication 100 is similar to duplication 136 discussed above, but it is longer than the latter since it includes in addition the *fu-cr* interval and the entire inert region (cf. Fig. 1).

The diploid females carrying duplication 100 are quite viable and fertile. They show the occipital bristles characteristics of most duplications for the *y-sc* region. The body build is somewhat longer and narrower than the normal diploid, the eyes narrower and quite rough. The wings occasionally show a notching on the inner margin, and the posterior crossvein is generally somewhat thickened. Males carrying the duplication are, on the contrary, quite inviable and completely sterile. The eyes are very rough, the occipital bristles present, the wings usually spread, with frequent notching, and the body build narrow, the abdomen being almost oblong in shape.

Since duplication 100 males seldom survive and are sterile, the usual method of obtaining intersexes carrying the duplication (*i.e.* crossing triploid females to duplication males) is inapplicable here. Fortunately, several triploid females carrying the duplication 100 were obtained in a diploid strain with this duplication, due to a spontaneous origin of triploidy. Such triploid females were mated to diploid males, and in their offspring twenty-two intersexes were found. Eleven intersexes were free from the duplication, and four of them belonged to the type II and seven to the type III. Eleven other intersexes carried the duplication (as shown by the suppression of the sex-linked recessives present in the stock), and these all belonged to the type VI, *i.e.* were practically female-like. No triploid females with the duplication were obtained in this generation, and consequently a duplication-carrying triploid stock could not be established.

Five out of the eleven intersexes with the duplication 100 were placed with normal males in individual cultures. One such culture proved fertile and produced thirteen diploid offspring and one male-type intersex. Some of the diploid females obtained carried the duplication, proving that the mother carried it too; moreover the appearance of the single intersex shows that the mother was triploid for the autosomes. It is,

therefore, proven that the addition of the duplication 100 to the chromosomal complement of an intersex transforms the latter in at least some cases into a fertile female. Since intersexes carrying the duplication 136 belong on the average to the type V (Table III) and are never fertile, the duplication 100 produces a stronger, and probably much stronger, shift toward femaleness than 136. Since, furthermore, the difference between these two duplications consists mainly in the presence of the *fu-cr* interval in the former and its absence in the latter, one must conclude that the *fu-cr* interval contains female modifiers.

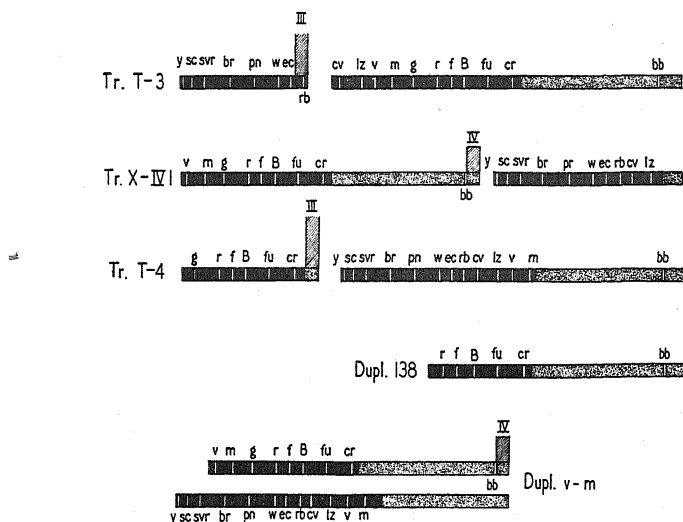


Fig. 2. Translocations used in the experiments described in the text. III, the third chromosome; IV, the fourth chromosome. The significance of other letters is the same as in Fig. 1.

Duplication 138 (undescribed), found by one of us, arose in the offspring of an X-ray treated Bar male mated to an \widehat{XX} female homozygous for vermilion, garnet, sable and forked. It appeared as a single vermilion, garnet, sable, Bar non-forked female. A further analysis showed that its origin is due to the X-chromosome of the Bar male being broken immediately to the left of the locus of rudimentary (between small-wing and rudimentary), the left part of the chromosome (*y*-small-wing) being lost, and the right part (rudimentary to bobbed) added as a free fragment to the chromosome complement of the \widehat{XX} female (Fig. 2). The length of the duplication 138 is equal cytologically to about one half of that of the normal X-chromosome. In the offspring of the original \widehat{XX} duplication females mated to *y-v-f-cr* male there appeared

a female whose origin must have been due to a crossing over between the duplication chromosome and the \widehat{XX} complex. The duplication is now attached to the spindle fibre end (the proximal or left end) of the normal X -chromosome, giving cytologically an unequal-armed V-shaped chromosome.

Females carrying duplication 138 are practically similar in appearance to normal females, except for the fact that they are somewhat smaller and their bristles are somewhat thinner. Their viability is lowered, and their fertility is very much below normal. Males carrying the duplication 138 mostly die, especially if the culture conditions are below optimum. If they survive they show, however, no striking somatic changes. They are completely sterile (about 100 of them were tested for fertility). In most of such males the genitalia, the anal tubercle and the internal parts of the reproductive system appear completely normal anatomically. In two males (found among several hundreds inspected) it was found, however, that one of their two anal plates was broken transversely into two separate plates. This finding is of importance. The structure of the anal plates (which are dorsi-ventral in females and lateral in males) is the first male characteristic to undergo a change in intersexes (Dobzhansky and Bridges, 1928; Dobzhansky, 1930*b*). Intersexes which depart even very slightly from the extreme male type have all the external male characteristics except for the anal plates which may be built as in females, or one of them may retain the male shape, and the other may be transversely broken in two. No normal males have so far been seen with such broken anal plates. It seems, therefore, justifiable to regard the broken anal plates as the first sign of intersexuality. The two duplication 138 males having this character are diploid intersexes. This fact alone is sufficient to show that the part of the chromosome involved in duplication 138 has strong female modifiers.

To obtain intersexes carrying the duplication 138, diploid females having the duplication were made homozygous for the third chromosome recessive gene discovered by Gowen (1931, 1933) which is known to disturb the chromosome pairing and to increase the frequency of formation of diploid eggs. These eggs fertilised by normal spermatozoa give triploid individuals. The presence of the duplication is easily recognisable in any individual since the duplicating fragment carries in this case the dominant gene *Bar* (see above). Triploid females were recognised by their usual characteristics (large eye-facets, lower density of hairs on the wing membrane). Several triploid females carrying the duplication were obtained by this method. They were mated to normal

males, and in their offspring eleven intersexes with the duplication 138 were found. Such intersexes (Fig. 3) are weak flies which hatch from

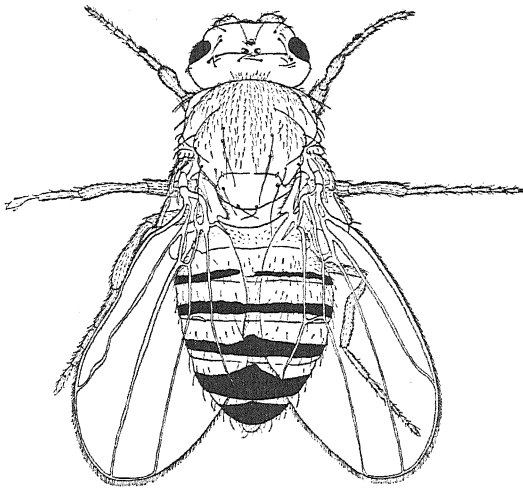


Fig. 3. A type V intersex carrying a duplication for the *r-bb* section of the *X*-chromosome.

the pupae with a great difficulty, so that many dead pupae in the culture bottles were found, upon dissection, to contain such intersexes. They must be classed as belonging to type V since all of them possess sex combs on both (or on at least one) of the front legs, and have all the other characters female. The wings are somewhat shorter and broader than normal and are not folded on the back in repose. The abdomen is somewhat more rounded and more shrivelled than in normal females, bristles are short and the Bar characteristics in the eye are exaggerated. The whole head is very small. A comparison of intersexes carrying the duplication 138 (Bar) and those free of it is shown in Table VI.

TABLE VI.

Type of intersexes in the cross 3N Dup. 138 B ♀ × wild type ♂.

	I	II	III	IV	V	VI	n	Mean type
Non Bar	15	10	26	—	—	—	51	2.22 ± 0.87
B	—	—	—	1	10	—	11	4.91

Dissection of the duplication 138 intersexes showed that the internal organs are female, but the ovaries are strongly underdeveloped, their condition being similar to that of not very extreme female-type intersexes. They must be sterile. One may conclude that the section of the *X*-chromosome involved in the duplication 138 contains more, or

stronger, female modifiers than that in duplication 136, but less than that in duplication 100. Indeed, duplication 100 intersexes are all of the type VI and a part of them are fertile as females, while duplication 138 intersexes belong to type V and are sterile.

Duplication 138 covers the region from rudimentary to bobbed; this region is longer than the right part of duplication 100, which includes only the region from fused to bobbed (Figs. 1, 2). As far as the right end of the *X*-chromosome is concerned these duplications differ from each other by the section containing the loci for rudimentary, forked, Bar and small-eye, which is included in 138 but not in 100. A part of this section, covering rudimentary, forked, Bar, but not small-eye, is involved in duplication 126 (Fig. 1). This duplication, induced by X-rays (Dobzhansky, 1932) represents a very short section of the *X*-chromosome, including only the loci of the three genes just mentioned, attached sidewise to the third chromosome, between the loci of scarlet and peach. Triploid females homozygous for forked were crossed to forked males carrying the duplication 126. Such males have the forked character incompletely suppressed. A part of the intersexes obtained in the offspring were semi-suppressed forked, and others were forked. The former carry the duplication, and the latter are free of it. A comparison of these intersexes is shown in Table VII.

TABLE VII.

Type of intersexes in the cross 3N forked ♀ × forked/Duplication 126 ♂.

	I	II	III	IV	V	VI	n	Mean type
Non-forked	8	26	17	21	1	—	73	2.74 ± 0.12
Forked	5	9	7	2	1	—	24	2.38 ± 0.21

The difference between the intersexes carrying the duplication 126 and those free of it is not statistically significant. It follows that either the section involved in this duplication is sexually neutral, or it carries only weak female modifiers.

DUPLICATIONS FOR THE *y-rb* AND *cv-bb* SECTIONS.

Mr J. Bonner found two translocations (unpublished) in both of which the *X*-chromosome was broken between the loci of ruby and crossveinless (Fig. 2). In one of these translocations, designated T-3, the yellow-ruby section is attached to the right end of the third chromosome, to the right of claret, and the crossveinless-bobbed section remains free. In the other, T-7, the *y-rb* section is attached to the right of speck in the second chromosome, and the *cv-bb* section remains likewise free.

Mr Bonner very kindly permitted the use of these translocations and of the above information for the purposes of the present study.

Both the T-3 and the T-7 translocation males produce four kinds of gametes: (1) containing both fragments of the *X*-chromosome, (2) containing the *y-rb* but not the *cv-bb* fragment, (3) the *cv-bb* but not the *y-rb* fragment, and (4) containing neither of these fragments. The *X*-chromosome involved in both translocations contains the wild type allelomorphs of the sex-linked genes. If a translocation male is crossed to females (diploid or triploid) homozygous for *y*, *cv*, *v* and *f*, four types of offspring, corresponding to the four types of gametes, should be produced. These types of offspring are: (1) wild type—carrying the broken chromosome and no duplications, (2) *cv v f* non-yellow—carrying the duplication for the *y-rb* section, (3) *y* non-*cv*, non-*v*, non-*f*—carrying the duplication for the *cv-bb* section, and (4) *y cv v f*—carrying neither the translocation nor the duplications. Offspring of the types (2) and (3) are of interest for us.

Non-yellow *cv v f* diploid females (*y-rb* duplication) are similar to females carrying duplications 112, 107, 118, 134 and 136 (see above), but the *y-rb* duplication, being longer than those just mentioned, produces a stronger effect. Occipital bristles are present in most flies, and the bristles on the wing-veins are also present in most cases. Wings are somewhat longer and narrower than in normal females, the bristles are heavier, eyes are distinctly rough, posterior crossveins are branched by a small extra vein extending into the second posterior cell at a right angle to the crossvein and disappearing after a short distance. Sometimes the second, third and fourth longitudinal veins are thickened and expand into little deltas near the margin. Some of these characters, especially the shape of the wings, of the bristles, and the roughness of the eyes, give the duplication-carrying females a considerable resemblance to the superfemales which show these characters in a more extreme form than the duplication females. Duplication females are fertile.

Non-yellow *cv v f* males are mostly inviable. Only four such males were found in the offspring of translocation T-3, and only two in translocation T-7, among thousands of males free from the duplication. Duplication males (Fig. 4) are small and abnormal flies which are completely sterile in spite of the fact that their genitalia and internal reproductive organs appear anatomically normal. The wings are broad and divergent, veins are thickened, bristles on the wing-veins and the occipital bristles are always present. The eyes are rough and the legs are frequently misshapen. Such males die usually in a few days after

emergence from the pupae. Diploid females and males carrying the *cv-bb* duplication were never found. They seem to be inviable. Translocation T-3 and T-7 males were crossed to triploid females homozygous for *y*, *v* and forked. Intersexes obtained in the offspring are shown in Table VIII.

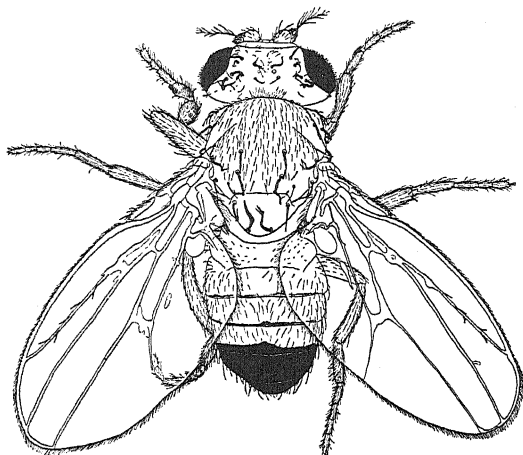


Fig. 4. A male carrying a duplication for the *y-rb* interval.

TABLE VIII.

Type of intersexes in the cross 3N *y v f* ♀ × Translocation T-3 and T-7 ♂.

	I	II	III	IV	V	VI	n	Mean type
T-3								
<i>y v f</i>	9	13	12	14	1	—	49	2.69 ± 0.16
Wild-type	34	56	43	39	—	—	172	2.51 ± 0.08
<i>v f</i>	—	—	—	—	6	9	15	5.60 ± 0.12
<i>y</i>	—	—	—	—	—	56	56	6.00
T-7								
<i>y v f</i>	16	20	22	19	2	—	79	2.63 ± 0.13
Wild-type	32	70	65	51	—	—	218	2.62 ± 0.07
<i>v f</i>	—	—	—	—	11	5	16	5.31 ± 0.13
<i>y</i>	—	—	—	—	—	153	153	6.00

Intersexes carrying the translocation (wild-type) are not different from those manifesting *y*, *v* and *f*. The *v f* intersexes (carrying the *y-rb* duplication) belong to the types V and VI. Their sexual characters are practically those of normal females (Fig. 5), but many of them have rudimentary sex-combs on one or on both legs (those having sex-combs on one leg only are included in type V). Their internal reproductive organs are female, but the ovaries produce no functional eggs, although they are subdivided into egg strings each consisting of several egg

chambers with growing oocytes—a feature not observed in the intersexes carrying duplication 136 (see above). The intersexes are sterile (about a dozen of them tested), and their viability is very low. Eyes are very rough, legs frequently misshapen, wings expanded but no occipital bristles and bristles on wing-veins were found.

Yellow intersexes (Fig. 6) are those carrying the duplication for the *cv-bb* section. They can also be described as triploid females having a deficiency for the *y-rb* section in one of their X-chromosomes. These

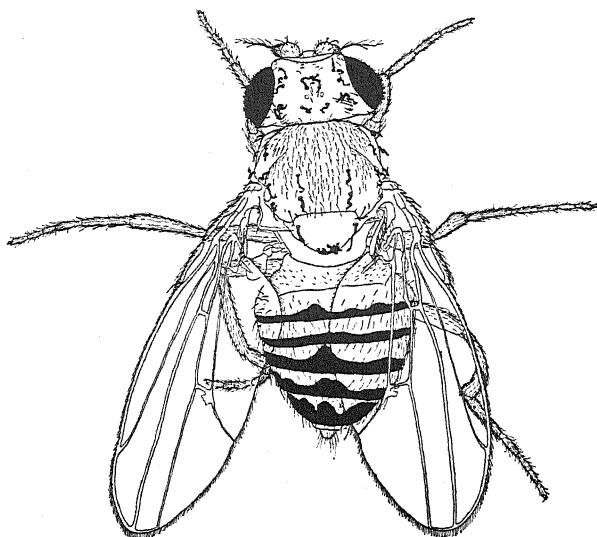


Fig. 5. A type VI intersex carrying a duplication for the *y-rb* interval.

intersexes are similar to triploid females (Fig. 7) but are smaller and their wings are frequently notched along the inner margin (Fig. 6). Postverticals and other bristles are frequently missing. Viability is good. About a dozen of such intersexes coming from the translocation T-3, and an equal number coming from the translocation T-7 were crossed to *y cv v B* males. About half of them proved fertile. The offspring of this cross appear in Table IX.

TABLE IX.

$y\ v\ f\ \widehat{XX}/cv-bb\ \text{duplication intersexes} \times y\ cv\ v\ B\ \delta$											
Diploid ♀			Triploid ♀		Intersexes			Males			n
<i>y v f</i>	<i>y v B</i>	<i>y B</i>	<i>y v B</i>	<i>y B</i>	<i>y v f</i>	<i>y v B</i>	<i>y B</i>	<i>y</i>	<i>y v</i>	<i>y v f</i>	
T-3	23	2	—	9	—	2	—	1	—	1	39
T-7	61	13	2	26	4	5	2	4	2	1	121

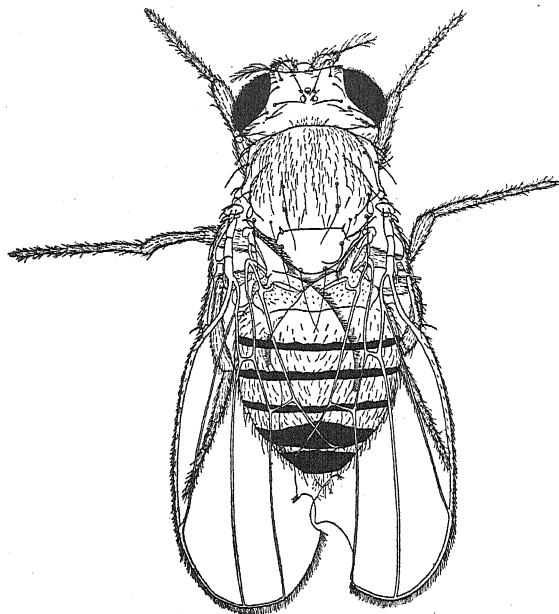


Fig. 6. A type VI intersex carrying a duplication for the *cu-bb* interval.

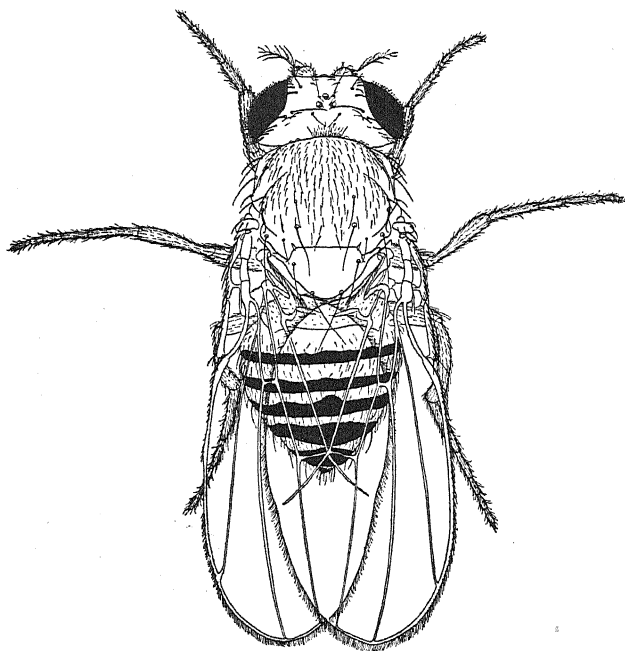


Fig. 7. A wild-type triploid female.

The appearance of $y v B$ and $y B$ diploid females, $y B$ triploid females, $y v B$ and $y B$ intersexes, and of all the males is due to crossing-over between the \widehat{XX} complex and the duplication chromosome¹. All the intersexes obtained do not carry the duplication, and belong to classes I, II and III. The results of these tests prove conclusively that the mothers of the cultures were actually duplication-carrying intersexes.

A comparison of the intersexes carrying the duplication for $y-rb$ and $cv-bb$ with those described above leads to the following conclusions. (1) Since intersexes carrying the $y-rb$ duplication are more female-like than those carrying duplication 136 (Tables 3 and 4), the $w-rb$ section of the X -chromosome carries female modifiers (duplication 136 is shorter than the $y-rb$ duplication since the latter includes the $w-rb$ interval and the former does not). (2) Since the $cv-bb$ duplication produces a stronger shift in the type of intersexes toward femaleness than duplication 138, the $cv-g$ section contains female modifiers.

DUPLICATIONS FOR THE $y-lz$ AND $v-bb$ SECTIONS.

In translocation X-IV 1 the X -chromosome has been broken between the loci of lozenge and vermilion, and between carnation and bobbed and the attachment of the spindle fibre. The vermilion-bobbed section has been attached to the fourth chromosome, and the yellow-lozenge section united with the spindle fibre attachment and remained free (Muller and Stone, 1930; Offermann and Muller, 1932; Muller and Painter, 1932; see also Fig. 2). Since the fly in which the translocation arose was wild-type except for the dominant gene *Bar*, the section attached to the fourth chromosome carries that gene. A male carrying the translocation produces four kinds of gametes: (1) carrying both fragments of the X -chromosome, (2) the $y-lz$ fragment (the portion of the chromosome including the spindle fibre attachment being disregarded since this portion represents the inert region), (3) the $v-bb$ fragment, (4) neither fragment.

Translocation T-IV 1 males (carrying *Bar*) were crossed to triploid females homozygous for y , v and f . The offspring obtained fell into four groups: (1) *Bar*, (2) $y v f$, (3) $v f$ non- y non- B , (4) $y B$ non- v , non- f . Class (3) carries the duplication for the yellow-lozenge section; class

¹ The $y v f$ triploid females used in this experiment had an \widehat{XX} complex homozygous for y , v and f , and a free $y v f$ X -chromosome. The duplication-carrying intersexes have, therefore, the \widehat{XX} complex and the duplication chromosome. The \widehat{XX} complex and the duplication chromosome in such intersexes seem always to disjoin at the reduction division and to pass to opposite poles. This explains the non-appearance of duplication carrying triploids, intersexes, and males other than crossovers.

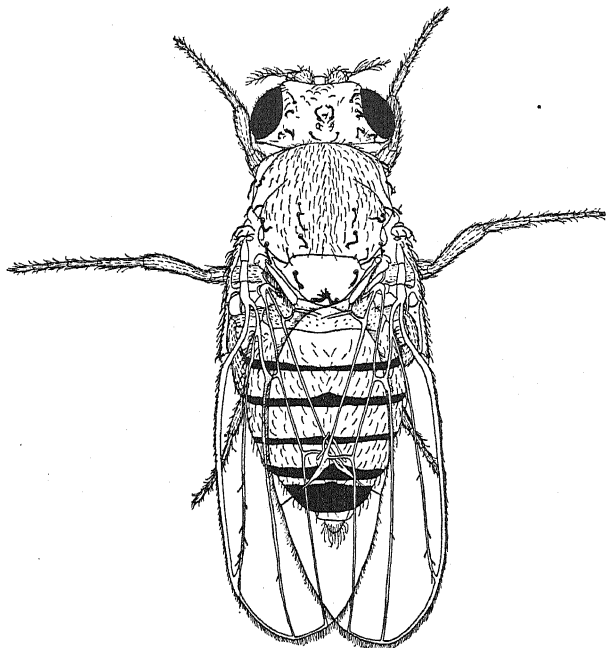


Fig. 8. Diploid female carrying a duplication for the *y-lz* interval.

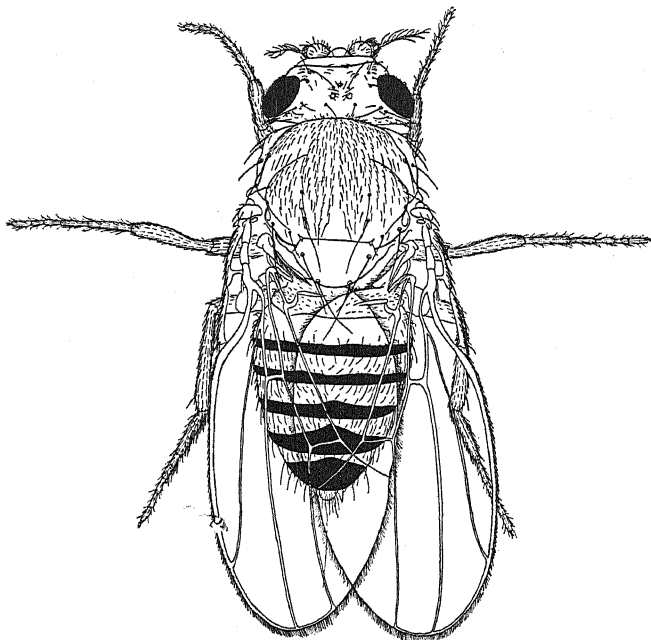


Fig. 9. Diploid female carrying a duplication for the *v-bb* interval.

(4) the duplication for the vermilion-bobbed section. As shown by Muller and Stone (1930) and Muller and Painter (1932), males carrying either duplication are inviable, but females survive and are sometimes fertile. We can confirm these observations. Diploid females with the *y-lz* duplication (non-*y*, non-*B*, Fig. 8) are rather similar to those carrying the *y-rb* duplication, but the departure from the females without duplication is more extreme here. Occipital bristles and bristles on the wing-veins are present, but the occipital bristles seem to be less strongly developed than in the *y-rb* duplication. Wings are long and narrow, sometimes misshapen, legs, especially the hind femora, are frequently bent, eyes are rough. All in all these duplication-carrying females may be considered as forms intermediate in appearance between females and superfemales. The yellow Bar non-*v* non-*f* females (duplication for the vermilion-bobbed section) are externally more similar to normal females than those carrying the yellow-lozenge duplication. The head and thorax are somewhat broader than in normal flies. The effect of Bar in the presence of this

TABLE X.

Type of intersexes in the cross 3N y v f ♀ × Translocation X-IV/♂.

	I	II	III	IV	V	VI	n	Mean type
<i>y v f</i>	36	48	37	11	—	—	132	2.17 ± 0.08
Bar	73	137	185	47	—	—	442	2.47 ± 0.04
Yellow Bar	—	—	—	—	1	64	65	5.98
<i>v f</i>	—	—	—	—	—	15	15	6.00

duplication is much weakened, the eyes being much broader than those of superfemales carrying Bar in single dose. The inner margin of the wing is sometimes scalloped. Postvertical bristles are occasionally missing. The intersexes obtained are shown in Table X.

The vermilion-forked intersexes (Fig. 10) carry the yellow-lozenge duplication. They are rather similar in appearance to the intersexes with the yellow-ruby duplication (cf. Figs. 5, 10) but the pathological characteristics (misshapen legs, expanded and crumpled wings) are even more extreme. All the fifteen individuals observed belong to the type VI, *i.e.* are more female-like than the intersexes with the *y-rb* duplication. Internal reproductive organs are, however, similar, and the ovaries are not sufficiently normal to produce functional eggs. These intersexes are completely sterile (about ten individuals were tested).

Yellow-Bar intersexes (Fig. 11) carry the vermilion-bobbed duplication. They are extremely female-like in appearance, and are distinguished from the duplication-carrying diploid females (Fig. 9) and from normal females by their smaller size, notched inner wing margin, more rounded

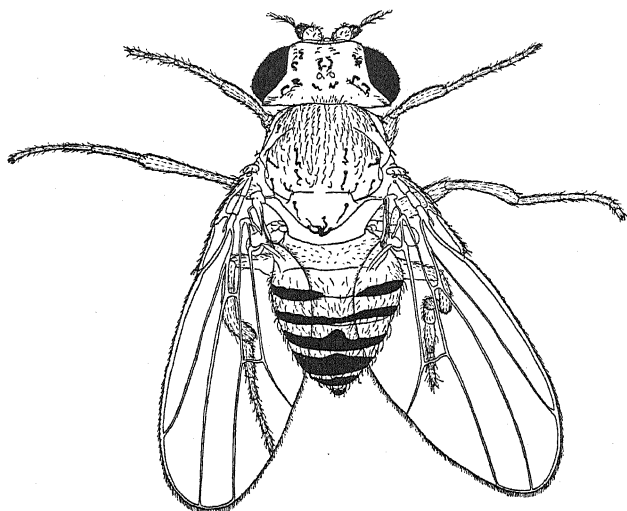


Fig. 10. A type VI intersex carrying a duplication for the *y-lz* interval.

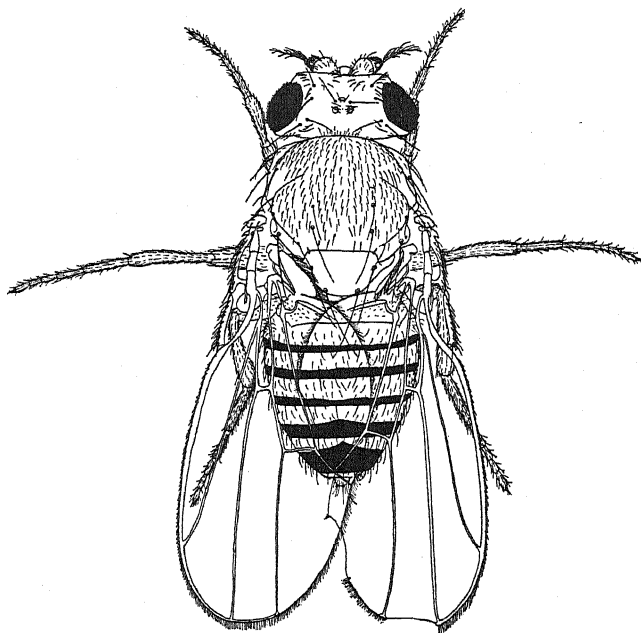


Fig. 11. A type VI intersex carrying a duplication for the *v-bb* interval.

abdomen, larger eyes with larger facets, and a lesser density of the hairs on the wing membrane. One individual had sex-combs on both legs (type V, Table IX) showing that these intersexes are not as much like females in their sexual balance as their appearance might suggest. The internal reproductive organs are female, ovarioles consist of several chambers with growing oocytes, but no eggs in advanced stages of development were found, and tests showed that these intersexes are sterile (about thirty individuals were tested).

Intersexes with the yellow-lozenge duplication are more female-like than those with the yellow-ruby duplication (cf. Figs. 1, 2, 5, 10, and Tables VIII, X). The crossveinless-bobbed duplication produces a greater shift toward femaleness than the vermilion-bobbed duplication (cf. Figs. 1, 2, 6, 11, and Tables VIII, X). It follows that the crossveinless-lozenge interval contains female modifiers (the locus of lozenge lies between *ct* and *v*, Fig. 1).

As shown by Muller and Stone (1930), diploid females carrying either of the two duplications are fertile. The present data add the information that neither of these two duplications produces fertile intersexes. This is important in its bearing on the possible existence of a "sex differentiator" in the *X*-chromosome. If such a factor exists it must be contained in either of the two fragments which constitute translocation *X-IV* 1. Hence one of the two types of intersexes should be fertile, and of the diploid females containing duplications one should, like a superfemale, be sterile. This is not the case. It must therefore be concluded that there is more than one sex-differentiating factor in the *X*-chromosome.

OLIVER'S T-4 TRANSLOCATION.

C. P. Oliver discovered a translocation between the *X* and the third chromosome (unpublished) which he calls the T-4 translocation. Prof. Oliver informs us that the nature of this translocation is as follows: the sable-carnation section of the *X* is attached to a third chromosome carrying the dominant *Dichaete* (*D*), and the yellow-miniature and bobbed sections are joined together and remain free (Fig. 2). In a cross of T-4 translocation males to triploid females homozygous for *y*, *v* and *f* four types of offspring are produced: (1) *D*, non-*y* non-*v* non-*f*, carrying the translocation, (2) *D y v* non-*f*, carrying the sable-carnation duplication, (3) *f* non-*D* non-*y* non-*v*, carrying the duplication for the yellow-miniature section, and (4) *y v f* non-*D*, free of the translocation and duplications.

Duplication-carrying males do not survive. Females with the sable-

carnation duplication (Fig. 12) are nearly similar to normal *Dichaete* females in appearance and differ only in a somewhat smaller size, thinner bristles and lowered viability and fertility. Females carrying the yellow

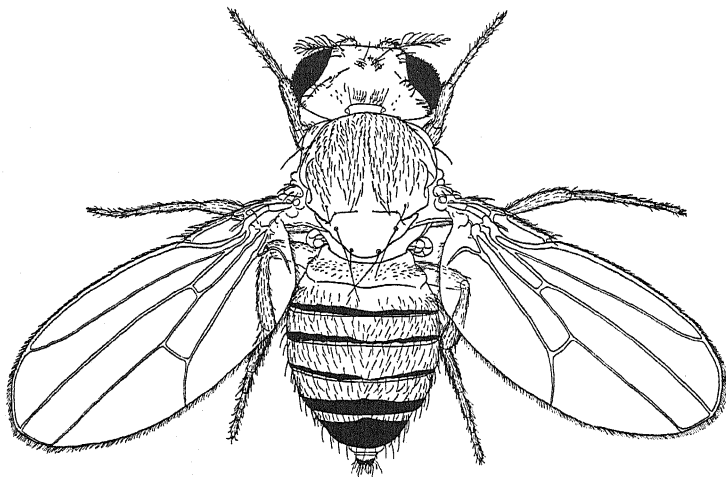


Fig. 12. Diploid female carrying a duplication for the sable-carnation interval.

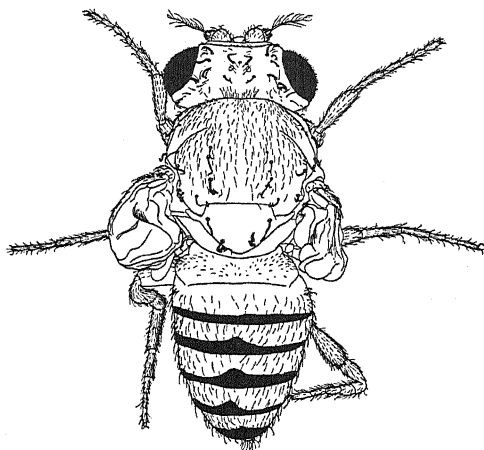


Fig. 13. A superfemale-like diploid female carrying a duplication for the *y-m* interval.

miniature duplication resemble superfemales almost entirely (Figs. 13, 14). This is not unexpected since the females carrying the yellow-ruby and the yellow-lozenge duplications resemble superfemales more closely than they do normal females. The viability of such females is very low, they are frequently unable to emerge from the pupa case. The wings

frequently do not unfold (Fig. 13), as is the case in many superfemales; sometimes the wings, however, develop normally and then have the narrow and elongate shape (Fig. 14) and notched inner margin characteristic of superfemales. Intermediate conditions are also frequent. Duplication females are mostly sterile, although one of the tested individuals has produced a few offspring. The ovaries, as shown by dissection, seldom

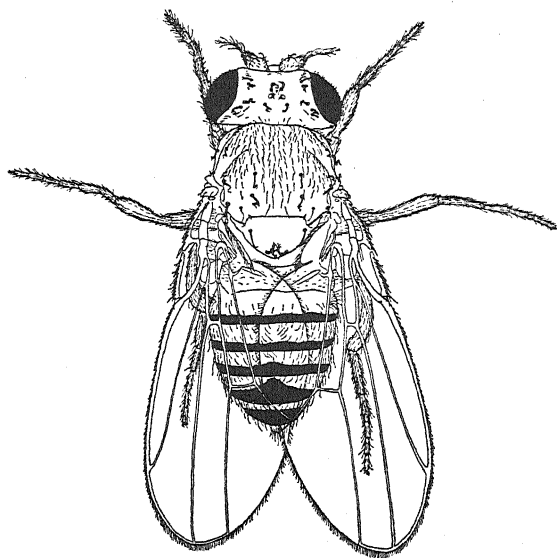


Fig. 14. A diploid female of the same genetic structure as that represented in Fig. 13 but having less abnormal appearance than the latter.

TABLE XI.

Type of intersexes in the cross 3N y v f ♀ × Translocation T-4 ♂.

	I	II	III	IV	V	VI	n	Mean type
<i>y v f</i>	54	46	22	22	1	—	145	2.10 ± 0.09
<i>D</i>	148	122	80	56	—	—	406	2.11 ± 0.05
<i>f</i>	—	—	—	—	—	121	121	6.00
<i>y v D</i>	—	—	—	—	—	7	7	6.00

contain one or two mature eggs, and usually contain none. The intersexes obtained are classified as shown in Table XI.

The yellow-vermilion-Dichaete intersexes (carrying the sable-carnation duplication) survive only rarely. This is the behaviour of duplication 138 (see above) which involves a rather similar region. Conversely, the yellow-miniature duplication, which is, as shown above, mostly lethal in diploid females, is quite viable in intersexes (the *f* intersexes, Table XI). Intersexes carrying either duplication (Figs. 15, 16) are extremely

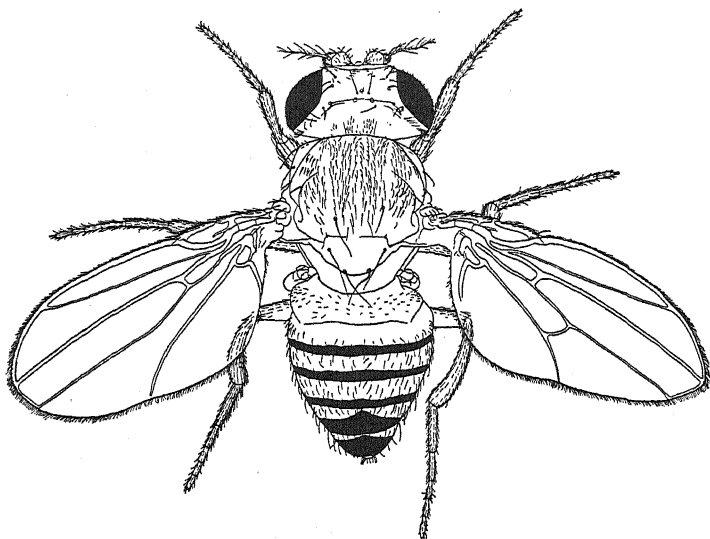


Fig. 15. A type VI intersex carrying a duplication for the sable-carnation interval.

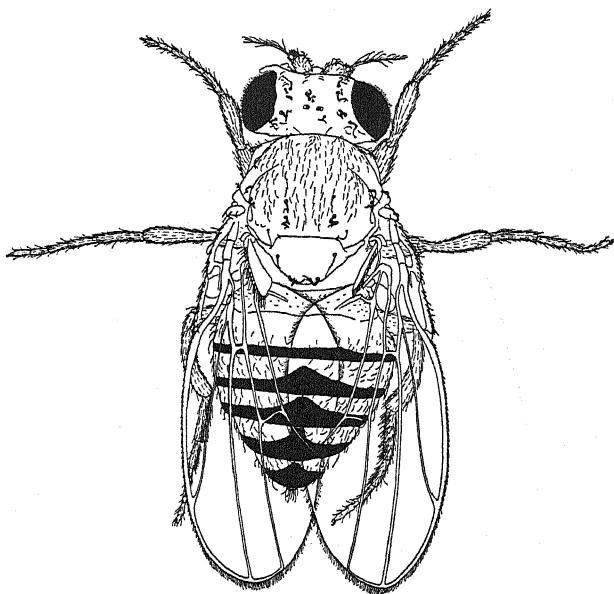


Fig. 16. A type VI intersex carrying a duplication for the *y-m* interval.

female-like, and all belong to the type VI. Those with the sable-carnation duplication have a shrivelled abdomen and are very weak. Four of them were tested for fertility and proved sterile; after more than a week during which they were allowed to lay eggs, they were dissected and their ovaries were found in a rudimentary condition. This may or may not mean that all the intersexes with this duplication are sterile, since the number of individuals obtained and tested by us is small.

The intersexes carrying the yellow-miniature duplication (Fig. 16) seem to be normal-looking and vigorous flies. In spite of this most of them are sterile and have rudimentary ovaries. Among approximately fifty individuals tested for fertility only a few (crossed to *y cv v B* males) produced offspring. Their offspring consisted of: $2N\ y\ v\ f\ \text{♀}$ —9; $2N\ v\ f\ \text{♀}$ —1; $2N\ f\ \text{♀}$ —1; *y v f* intersexes (male type)—2. The production of intersexes proves the intersexual nature of the individuals tested. We may remark here that the distinction between the duplication-carrying intersexes and the corresponding classes of the duplication-carrying diploid females is sufficiently reliable even in cases when the individuals are sterile and their offspring cannot, consequently, be obtained. Intersexes always have larger eye facets and sparser hairs on the wing-membrane than diploid females.

DUPLICATIONS FOR *v*, *m* AND *g*.

As a direct result of chromosome breakage, no stocks carrying duplications for the vermilion-miniature region (Fig. 1) have so far been established¹. This region is included in some of the duplications described above, but each of these duplications contains also a long section lying to the right or to the left of the vermilion-miniature one. By the combination of the proper fragments from translocation X-IV 1 and Oliver's T-IV translocation, a duplication for the vermilion-miniature region was prepared. It will be remembered (Fig. 2) that one of the fragments of

¹ Such duplications have, however, been obtained. Wild-type males treated with X-rays were crossed to $\bar{X}\bar{X}$ females homozygous for vermilion, sable, garnet and forked. In the offspring there were found one forked non-vermilion non-sable non-garnet female, two vermilion non-sable non-garnet non-forked females, and one vermilion forked non-sable non-garnet female. These individuals, as shown by their phenotype, must have carried duplications for the whole or a part of the vermilion-garnet region. They were crossed to *y v f cr* males, and each of them produced a small number of offspring among which, however, no duplication-carrying flies were found. Stocks containing duplications could not, consequently, be established. Nevertheless, the fertility of the original duplication females is significant. It shows that the sections of the chromosome involved in these duplications do not contain sufficiently strong female modifiers to transform the duplication-carrying females into sterile superfemales.

translocation X-IV 1 contains the loci *v-bb*, while a fragment of translocation T-4 contains the loci *y-m*. An individual containing these two fragments substituted for a normal X-chromosome therefore contains in duplicate the *v-m* region.

By crossing females heterozygous for eosin Bar translocation X-IV 1 eosin bobbed-lethal to Oliver's T-4 translocation males, it was possible to detect this duplication. The duplication is contained in any non-eosin Bar male (cf. Fig. 2). Such males were found, and it was found in addition that the females carrying the same duplication could easily be distinguished by the broadness of the Bar eye in the presence of the duplication.

Females containing the *v-m* duplication are practically normal in viability and fertility. In their appearance no signs of any attributes of the superfemales are noticeable¹. Males carrying the same duplication are rather stockily built, with short wings, having some resemblance to miniature, bristles and wing-veins are normal. These males were tested for fertility and found to be absolutely sterile. Since their sterility might be suspected to be due to the absence of the Y-chromosome (and XO males are known to be sterile) a different experiment was carried out. The broad-Bar duplication-carrying females were crossed to normal males. In the offspring of this cross half of the males should carry the duplication, and they must also carry a Y-chromosome. Such males were actually obtained. An inspection of their morphology, however, showed that their genitalia are abnormal, much in the same way as in the triploid intersexes. In some of them the external genitalia are asymmetrical, the genital arch and the penis being more or less rudimentary. The internal reproductive organs show the same features as observed in type II intersexes: testes are frequently cocoon-shaped instead of spiral, sometimes only one instead of two vasa efferentia and accessory glands are present. Many individuals have even no external genitalia and no anal tubercle, resembling, therefore, intersexes of the type III. This resemblance is, however, in most cases only superficial for they have misshapen male genitalia inside of the body. This phenomenon is found, although rather seldom, in triploid intersexes and even in normal males. It is due to a failure of the imaginal disc forming the genitalia, the anal

¹ The Bar eye is very broad in these females, much broader than the usual heterozygous Bar, although not so broad as in a duplication for the whole *v-bb* fragment of translocation X-IV 1. In the presence of the duplication, the sex-linked recessive scute evinces dominance in the post-vertical bristles. The males carrying the duplication also show a broadening of Bar eye. The interest of these data from the point of view of genic balance is obvious.

tubercle and the vas deferens to evert. Real type III intersexes have a different structure of the genitalia region: the imaginal disc of the genitalia is rudimentary, but the anal tubercle is present, being built according to the female plan. Finally, some of the duplication-carrying males had apparently normal external and internal genitalia, being, thus, similar to intersexes of the type I. These males were tested for fertility and found to be sterile. The frequency of the different types of the structure of the reproductive organs in the duplication-carrying males is shown in Table XII (the classification applied is the same as that applied to triploid intersexes; individuals having no external genitalia and no anal tubercle being included in type III).

TABLE XII.

Intersexuality in males carrying the duplication for v-m cr-bb sections.

	I	II	III	IV	V	VI	n
First experiment	55	11	99	—	—	—	165
Second experiment	20	—	—	—	—	—	20

Why did the *v-m* duplication produce no obvious signs of intersexuality in the first experiment and yet produce them in the second? It seems reasonable to assume that this difference is due to the genic modifiers which might have been different in the two experiments. Such shifts in the degree of intersexuality are frequently observed when triploid females are crossed to males from different strains. It is remarkable that the *v-m* duplication transforms males into what may be called diploid intersexes, but females carrying the same duplication are nearly normal in appearance and seemingly normal in fertility. At any rate this duplication cannot be assumed to include the locus of the hypothetical sex-differentiator since its presence in females does not change the latter into superfemales, nor does its presence in males change them into females. Note added in proof: Females carrying a corresponding deficiency have recently been studied. They showed not the slightest sign of intersexuality.

DISCUSSION.

According to the concept of genic balance (Bridges 1922, 1925), the effect of any chromosome on the sexual development of an individual is a net effect, the resultant of the interaction of the male and female modifiers in that chromosome. Within any given small section, a balance quite different from that of the whole chromosome may be found.

In the X-chromosome, with which we are chiefly concerned here,

Bridges has shown that the net effect is in the female direction. The experimental study of how the sex factors are distributed in this chromosome was made possible following the production of chromosome fragments of different lengths due to X-ray treatment. The changes in sexual development which are induced by the addition of such fragments to otherwise normal individuals may be observed, and in this way the net effect of the modifiers for sex in any given fragment may be measured. Painter and Muller (1929), Muller (1930*a*, 1932) and Muller and Painter (1932) were first to study the effect of a large number of such duplicating fragments on the sexual development of otherwise normal males and females. They found that the additions of sections containing portions of the left end and of the right end of the *X*-chromosome were relatively ineffective in changing the sexual characteristics of an individual. This indicated either that the modifiers in those sections were too weak to be effective, or that no female modifiers were present.

The extensive and careful studies of Patterson (1930, 1931) carried the analysis of the problem still further. In the progeny of flies treated by X-rays, he found mosaic individuals having in some parts of the body two normal *X*-chromosomes, and in the others one normal and one fragmented *X*-chromosome. The parts of the mosaics containing the two normal *X*-chromosomes were, of course, always females. But those containing the normal *X* plus the fragment were of either sex, depending on the nature of the fragment. Patterson found that losses of sections of the left end of the chromosome produce no changes in sex, unless the sections lost are longer than the yellow-vermilion interval (Fig. 1). In such cases—the loss, for example, of the yellow-forked section—the parts carrying the small remaining fragment are male and the mosaic individual is a gynandromorph. On the basis of these data, Patterson concluded that “if there is a gene for sex, it must lie in that portion of the chromosome occupying the middle region, at some point between the loci of singed and forked or their normal allelomorphs.” Patterson emphasises the tentative nature of this conclusion.

It seemed to us that a study of the intersexes carrying duplications and deficiencies should give more exact information regarding the distribution of the sex factors than a study based on duplication-carrying females and males. As shown by Dobzhansky (1930*a, b*), relatively minor variations of the sexual balance produce easily noticeable changes in the type of intersexes; but the same variations show no effect at all on females or males. The reason for this is clear: the balance of intersexes, between the male and female, can easily be shifted in either direction. But the

normal sexes are so far to either side, their specific balance so well insured, that these minor variations are rendered ineffective by the presence of this "margin of safety." In developmental terms this is tantamount to saying that the threshold for change is much higher in the normal sexes than in intersexes. This means that intersexes provide a much more sensitive indicator of the presence of sex factors in a given portion of chromosome than do the normal sexes.

These considerations are justified by the data presented above. It appears that female modifiers predominate in all parts of the *X*-chromosome, with the single exception of the inert region, which seems sexually neutral. The presence of the duplications for all sections of the *X*-chromosome studied shifts the type of intersexes toward femaleness. Only in the case of the rudimentary-forked-bar duplication (duplication 126, Fig. 1 and Table VII) the effect produced is not statistically significant. But this duplication, it will be remembered, is probably the shortest in our material. And with increasing length of the active portion of the *X*-chromosome, the shift of the type of intersexes towards femaleness becomes greater and greater. Thus, duplications for *y*, *sc*, and *svr* (duplications 107, 112, 118, Tables III, VII) produce a marked increase on the proportion of female type intersexes. With the addition of the broad locus (duplication 134, carrying *y*, *sc*, *svr* and *br* Tables III, VII) this effect is intensified. A still greater effect is produced by duplication 136 (*y-pn*), and by the duplications for the *y-rb* interval. Intersexes containing a duplication for the *y-lz* interval (Table X) are to all appearances simply sterile females. And finally, these are fertile when the duplication added includes the interval *y-m*.

A similar series is available if duplications for the right end are considered. Intersexes carrying a duplication for *r-bb* belong, on the average, to class V. In the presence of *v-bb* duplication, they belong to class VI, but are still sterile. And last, intersexes are changed into fertile females in the presence of a *cv-bb* duplication.

Are these data consistent with the assumption of a single sex-differentiator? This would require that the addition of a single short region of the *X* transform an intersex into a fertile female. Clearly this is not the case. Intersexes containing either the *y-pn* interval (duplication 136) or the *fu-bb* interval (duplication 138), are sterile. The addition of the duplication 100, where these two intervals are combined (*y-pn*, *fu-bb*) produces a fertile individual. Similarly for the regions *cv-ct*, *v-bb* (Fig. 2) and *y-lz*, *v-m* (Fig. 2). No single region avails to produce fertility; rather we must suppose that the length of the sections added, with the

single exception of the inert region, is of importance. The assumption of a specific sex-differentiator is therefore neither necessary nor sufficient to account for the data.

The relative effectiveness of the modifiers in different sections of the chromosome is another question. If all modifiers were equally effective, the sex determining power of a fragment of chromosome would be a direct function of its length, within limits, again disregarding the inert region. This cannot as yet be answered in complete detail. The data are inadequate to define the cytological map of the *X* sufficiently accurately, hence exact data on the relative lengths of the different fragments are not available. Yet the existing information shows on the whole a correlation between length of fragments and effectiveness. Some indications of minor variations in the strength of the sexual modifiers have been found. Duplication 126 produces no significant effect on intersexes. This may indicate, in spite of the small size of the fragment, that very weak modifiers are involved, or that the fragment is sexually neutral. On the other hand, the *v-m* duplication makes diploid males intersexual. This suggests that the female modifiers in the section involved are especially strong. Patterson's data on mosaics point in the same direction. It is this region, in addition to that from *y* to *v*, which produces a sex reversal in mosaics. Unfortunately this region is precisely the one least studied cytologically (Muller and Painter, 1932; Dobzhansky, 1932*a*). Moreover, we have not as yet studied the effect of this duplication on intersexes. This is necessary, since the duplication 138 (*r-bb*) occasionally produces intersexual characteristics in males, yet has no unwarrantedly strong effect on intersexes. It should be remembered that most long duplications make males inviable so that their effects on the sexual characteristics of males cannot be directly studied.

The effect of the *X*-chromosome of *Drosophila melanogaster* on sex-determination is then the joint effect of many, more or less equivalent genes, as Bridges originally supposed. What is the relation of this to the general problem of sex differentiation? The supposition (Muller, 1932, Darlington, 1932) that the original change from a hermaphrodite to a dioecious species occurred as a result of a single gene change seems quite plausible. Indeed, in organisms in which this is a rather recent phenomenon, such as the lower vertebrates, the genetic evidence indicates that a gene mutation may produce precisely such effects. The work of Winge (1932) on *Lebistes*, of Kosswig (1933) on *Xiphophorus*, or of Witschi (1929) on different races of *Rana* need but be mentioned.

In the insects, however, a somewhat different phylogenetic situation

exists. There exist a single species of hermaphrodite insects, and it is probably not "ancestral." The group is adjusted to a dioecious existence, and on the general principle of a margin of safety, a polymeric determination of sex would be of selective advantage, since mutation at a single locus would no longer be so effective in disturbing the sexual balance. From this point of view, it seems quite reasonable that a large group of modifiers be segregated in the *X*-chromosome, whose quantitative relation to the autosomes determines sex. Since this quantitative relation is safeguarded by the general stability of the mitotic mechanism, such a polymeric system offers the most stable system of sex determination, and it is not surprising that it should actually be found. There are some indications in the *Lymantria* work that this may also be true here: the nature of the "T" modifier (Goldschmidt, 1923) which changes intersexes in *Lymantria*, may be relevant to our present discussion.

In general, it should perhaps be emphasised that which gene in any specific case is the sex differentiator is very likely a matter of chance. The sexual development of an individual is a matter involving many threshold reactions, and an effect on any one of them, the others being constant, may be sexually differentiating. But it does not follow that the particular effect in question is of most importance in sexual development. It was not only the last straw that broke the camel's back.

SUMMARY.

1. The problem of the distribution of the sex-determining genes in the *X*-chromosome of *Drosophila* is attacked by studying the sexual characteristics of females, males and intersexes carrying duplications and deficiencies for various sections of that chromosome.

2. Intersexes are especially favourable material for such studies since even small variations in their sexual balance produce clearly noticeable changes of the sexual characters. Similar variations in the sexual balance of normal females and males produce no visible effect.

3. Duplications and deficiencies for the inert region do not affect the type of intersexes. The inert region of the *X*-chromosome is neutral with respect to the sexual balance.

4. Duplications for any section of the chromosome studied, except the inert region, produce a shift toward femaleness in the average type of intersexes. The *y-sc* deficiency, on the contrary, produces a shift toward maleness.

5. The extent of shift in the female direction produced by various duplications is on the whole proportional to their cytological lengths. Whether an exact proportionality obtains in all cases cannot be decided at the present, although some facts indicate that minor variations in this respect are observed.

6. Intersexes carrying duplications for sufficiently long sections breed as fertile females. There is no single locus in the chromosome the presence of which in triplicate is necessary for the fertility of the intersexes.

7. Long duplications (for *r-bb*, and for *v-m* intervals) cause the appearance of intersexual characters in diploid males. Other long duplications (*y-rb*, *y-lz*, *y-m*) give to females an appearance similar to that of superfemales.

8. The sex-determining rôle of the *X*-chromosome of *Drosophila* is due to a co-operative effect of numerous female modifiers located in all regions of the chromosome except the inert region.

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A CYTOLOGICAL STUDY OF THE GENUS *SORGHUM* PERS.

II. THE MEIOTIC CHROMOSOMES.

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(With Twenty-seven Text-figures.)

IN the first section of this paper (1932) an account of the somatic chromosomes of the section *Eu-sorghum* of the genus *Sorghum* Pers. was presented. In this section meiosis in pollen mother cells of some of the species will be described. The material is the same. Spikelets from the plants grown at the John Innes Horticultural Institution, Merton, in 1930, under the conditions described in the preceding section, were fixed for 1 min. in Carnoy's 6:3:1 fluid and then for 24 hours in La Cour's 2Bd, the end of the glumes having previously been clipped off to facilitate penetration. After embedding in paraffin, sections were cut at 16μ , and stained by Newton's iodine gentian-violet method. Drawings were made at the same magnification as those in the preceding section but are reduced to $3000\times$ in reproduction.

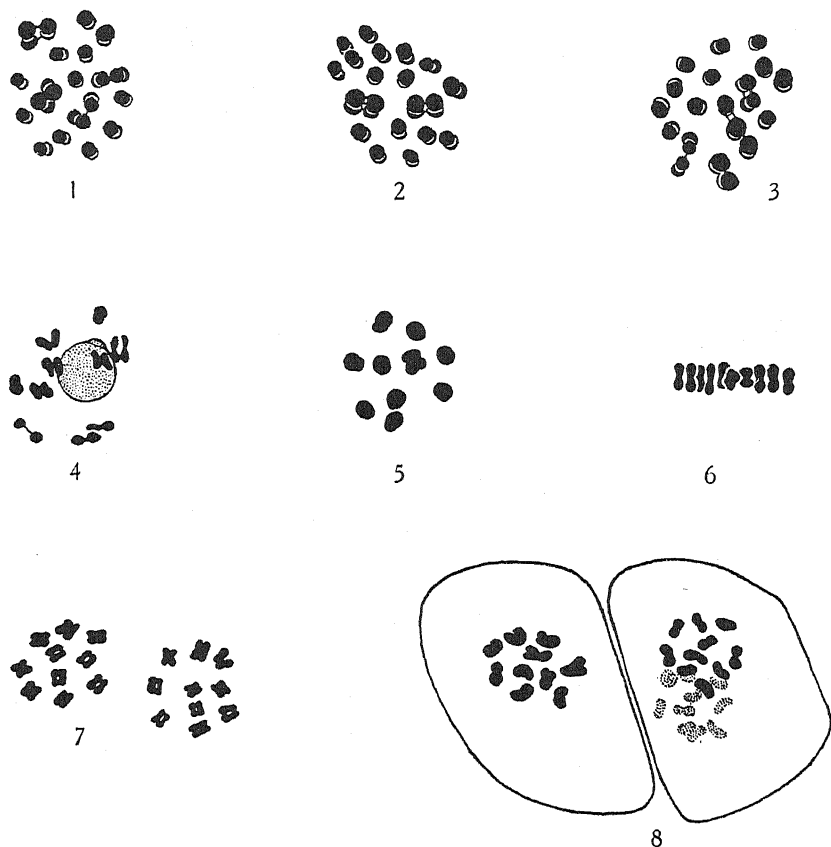
OBSERVATIONS.

Meiosis has been studied in practically all of the species and varieties from which observations of mitosis have been recorded in the preceding section, but since many of them exhibit no distinctive features, only a few are here illustrated.

In *S. halepense* there are most commonly from 10 to 14 bivalents, and the remainder of the 40 chromosomes are in quadrivalent or higher associations. In Fig. 1 there are $12_{II} + 4_{IV}$; in Fig. 2 $16_{II} + 2_{IV}$; in Fig. 3 $11_{II} + 3_{IV} + 1_{VI}$.

In all the diploid forms of *Sorghum* examined, 10 bivalents are most commonly formed, but quadrivalent associations are also common and sexivalents are found occasionally. Fig. 4 represents a diaphase in *S. sudanense*, in which there are 10 distinct bivalents: and Fig. 5, a metaphase of *S. cernuum*, also having 10 distinct bivalents. Fig. 6 is a 10-bivalent metaphase of *S. cernuum* seen in side view. Fig. 7 is a polar view of a heterotypic anaphase of *S. virgatum*. (The slide was moved after the

upper plate had been drawn so that the lower plate should not be superimposed upon it.) A homoeotypic division in *S. sudanense* is shown in Fig. 8, one of the daughter cells being at the metaphase and the other at late anaphase.

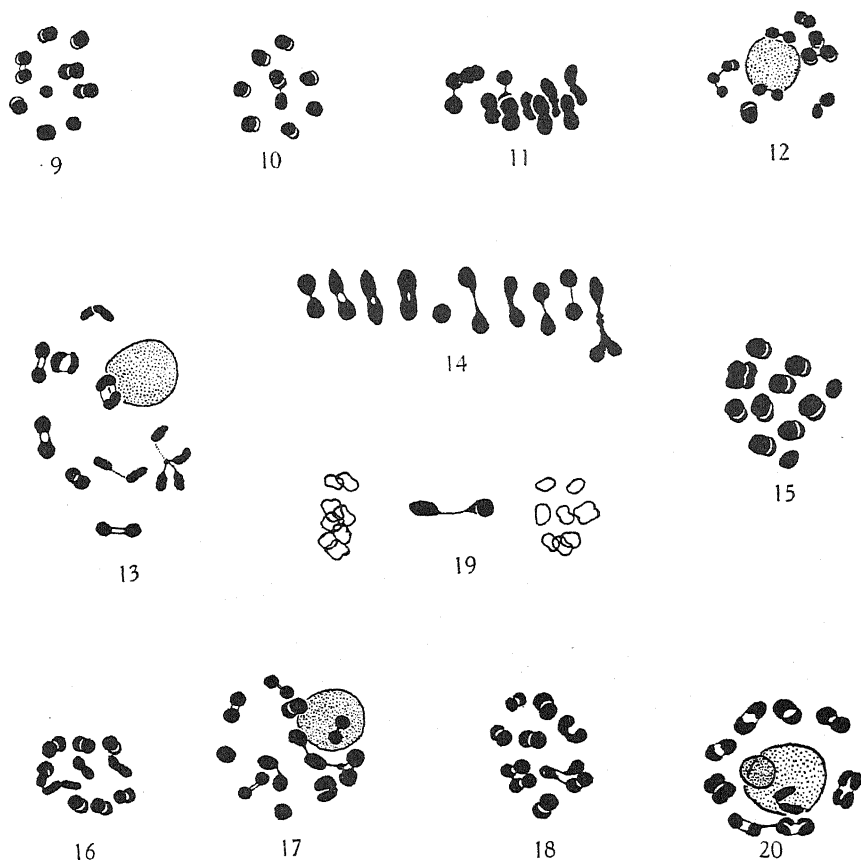


Figs. 1-8.

Figs. 1-3. *S. halepense*. Figs. 4 and 8. *S. sudanense*. Figs. 5 and 6. *S. cernuum*.
Fig. 7. *S. virgatum*.

Figs. 9-20 illustrate multiple configurations in pollen mother cells of diploid species. In Figs. 9 and 10, polar metaphase views of *S. margaritifera*, the 20 chromosomes are seen to be associated as $8_{II} + 1_{IV}$ and as $7_{II} + 1_{VI}$ respectively. Fig. 11 represents a side metaphase view of the same species, in which there are $8_{II} + 1_{IV}$. The associations found at late diaphase in one cell of *S. cernuum*, shown in Fig. 12, are $5_{II} + 1_{IV} + 1_{VI}$. Figs. 13, 14 and 15 are from *S. subglabrescens*. In the middle diaphase cell

shown in Fig. 13 there are $8_{II} + 1_{IV}$; in the side view anaphase of Fig. 14 (the chromosomes being spaced out for the sake of clearness) there are $8_{II} + 1_{III} + 1_I$; and in the polar view metaphase of Fig. 15 there are $9_{II} + 2_I$. Fig. 16 shows $8_{II} + 1_{IV}$ in a polar metaphase view of *S. melaleucum*.



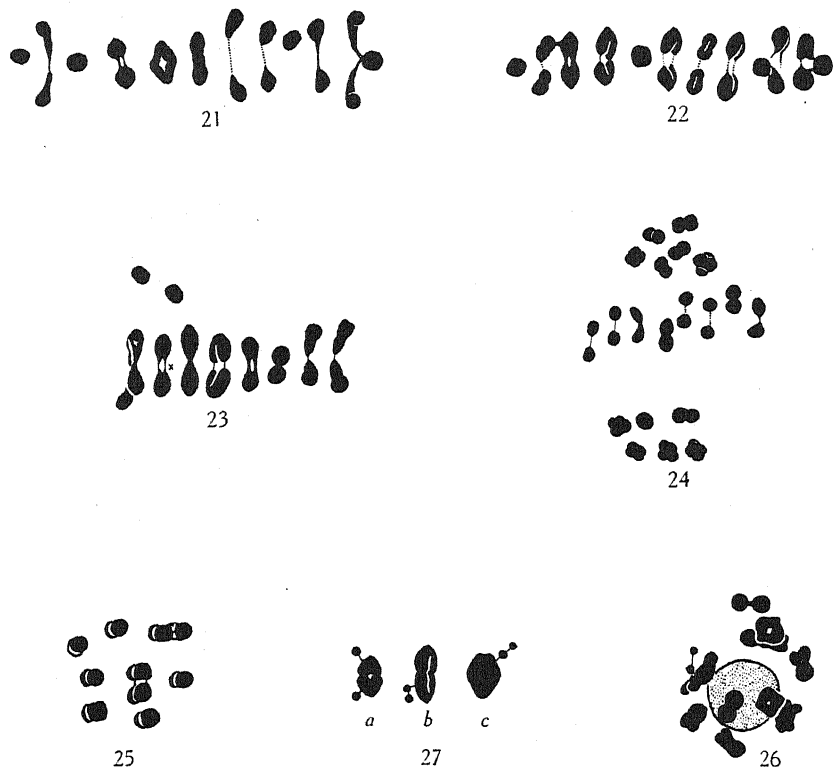
Figs. 9-20.

Figs. 9-11. *S. margaritifera*. Fig. 12. *S. cernuum*. Figs. 13-15. *S. subglabrescens*.
Fig. 16. *S. melaleucum*. Figs. 17-19. *S. durra*. Fig. 20. *S. sudanense*.

leucum. In Fig. 17, a very late diaphase of *S. durra*, there are $7_{II} + 1_{IV} + 2_I$. In Fig. 18, a polar metaphase of another strain of *S. durra*, there are $6_{II} + 2_{IV}$, and in Fig. 19, a heterotypic anaphase of a third strain of *S. durra*, eight chromosomes have reached each pole, and a quadrivalent is just dividing at the equator. In Fig. 20 a middle diaphase of *S. sudanense*, there are $6_{II} + 2_{IV}$, the constituent chromosomes of one

of the two quadrivalents being loosely, and those of the other closely associated.

Figs. 21-4 are all from Dakota Amber Sorgho, Figs. 21-3 being heterotypic metaphases and Fig. 24 a heterotypic anaphase. In all of these four figures, the chromosomes have been spaced in drawing. In Fig. 21 there are $7_{II} + 1_{III} + 3_I$. In Fig. 22 there are definitely



Figs. 21-27.

Figs. 21-24. Dakota Amber Sorgho. Figs. 25-27. *S. verticilliflorum*.

$4_{II} + 2_{III} + 2_I$. In addition there is possibly a quadrivalent, since there appears to be a connection between the two pairs of chromosomes on the left. But we do not think this is a real union and consider that the complement of this cell is almost certainly $6_{II} + 2_{III} + 2_I$, not $4_{II} + 1_{IV} + 2_{III} + 2_I$. In Fig. 23 there are $7_{II} + 1_{IV} + 2_I$, and in Fig. 24 there are 6 chromosomes in each anaphase plate and 8 univalents dividing at the equator.

In this plant of Dakota Amber Sorgho there are univalents in at least 20 per cent. of the cells, their numbers ranging from 1-16, the mode

being about 4, and the multivalents are much more common than in any other variety. It is difficult to state even an approximate percentage of occurrence, since in many cells there are apparent multivalents which cannot positively be identified as such, but perhaps 10 per cent. of all cells clearly seen would be a reasonable estimate of the proportion showing multivalents.

Figs. 25-7 are from a plant of *S. verticilliflorum* having an additional pair of fragments. Fig. 25 is a polar view of the heterotypic metaphase showing $6_{II} + 2_{IV}$. The fragments could not be seen in this cell. Fig. 26 is a late diaphase in which there are $10_{II} + 1_{II}$ ff. The paired fragments, though lying against a bivalent in the drawing, are actually well away from it in a different focal plane. Figs. 27 *a*, *b* and *c* are bivalents from three different heterotypic metaphase plates, each having the pair of fragments attached. In Fig. 27 *a* one fragment is attached to each member of the bivalent. In Figs. 27 *b* and 27 *c* the fragments are paired and only one of them attached directly to the bivalent.

Secondary associations are very common in all the species, but since it is always difficult to distinguish real secondary pairing from accidental juxtapositions, and definite multivalents are relatively so common in *Sorghum*, only the latter have here received serious consideration.

DISCUSSION.

The occurrence of multivalent chromosome associations in all the "diploid" species of *Sorghum* studied, and of associations higher than quadrivalent in the "tetraploid" *S. halepense*, obviously raises many points of genetic interest. These have significance for other genera also, since ten has commonly been considered the basic chromosome number of the Andropogoneae and Maydeae. They must especially be considered in relation to *Zea Mays*, since it has been so extensively investigated cytogenetically and is in so many respects similar to *Sorghum* (cf. Karper 1931 and Karper and Conner, 1931).

In maize, multivalents have been reported only in cases where cytogenetic evidence showed them to result from either reciprocal segmental translocation or simple translocation followed by duplication. But Beadle (1931) shows occasional pairing in the haploid pollen grain divisions of polymitotic maize.

The multivalents of *Sorghum* can scarcely be due to translocation, unless, as is extremely improbable, all the translocations necessary to explain the observations have been advantageous ones, for *Sorghum* is about 94 per cent. self-fertilised (Karper and Conner, 1931) and they

would therefore continually be eliminated from the population (cf. Brink and Burnham, 1929).

The observation of additional paired fragments in one plant of *S. verticilliflorum* also bears on the general problem, since tetrasomy commonly has a greater effect in diploids than polyploids. There were no obvious differences in either the morphology or fertility of this plant. Variations in chromosome number and supernumerary fragments occur likewise in maize.

The occurrence of duplicate or polymeric factors, as defined by Bateson (cf. Tjebbes, 1931) may, with certain reservations, be taken as an indication of polyploidy. Sprague (1932) cites ten cases of duplicate genes and three of triplicate in maize. Though relatively few studies have yet been made on *Sorghum*, duplicate genes for peduncle shape are known (Hayes and Garber, 1927), and Karper and Conner (1931) have found indications of polymeric genes governing chlorophyll development in *S. sudanense*. Though, as shown by Sprague, the "residual genetic mass" may determine whether a pair of factors will produce 9 : 7 or 15 : 1 ratios, the relative frequency of duplicate factors in maize and *Sorghum* agrees with the cytological evidence that 10 is not their basic chromosome number. In *Sorghum* there is more direct evidence in the discovery by Karper (1930) of a 5-chromosome species, *S. versicolor*, though he states that its chromosomes appear like "tetrasomes rather than disomes."

Fewer than 7 units of association were not found in our *Sorghum* material. This, together with the frequency with which the chromosome number 7 and its multiples occurs in the Gramineae, raises the possibility of it, rather than 5, being the basic number. It may be noted that in *Oryza sativa*, having 12 pairs of chromosomes, 5 independent groups of polymeric factors are known (Chao, 1928), and that in it Kuwada (1910) has recorded "secondary pairing" at metaphase II, and we have observed multivalents at metaphase I. Again, though multiples of 10 are characteristic of *Saccharum*, as of the Andropogoneae in general, Bremer (1932) has found a Java clone of *Saccharum spontaneum* and also *S. biflorum* from North Africa to have 56 chromosomes.

The multivalent formation in *Sorghum*, though not frequent enough seriously to disturb most genetic studies, would be expected to produce chromosome mutations. Where these involve only small segments of chromosomes they will often, in the absence of detailed analyses by linkage tests, pass for gene mutations. The mutation rate for a certain "gene" in *Sorghum* is considerably higher than in any in maize (Karper, 1932), and there is evidence that this applies to a number of "genes"

(Karper and Conner, 1931). This would be expected from our observations, if they are really duplications or deficiencies, or reverse changes from such conditions. The differences in linkage values found in different strains of maize by Stadler (1926) and Collins and Kempton (1927), and especially the latter's observation of lower crossing-over in plants heterozygous for *R*, are, together with observations of pairing within the haploid chromosome set of maize, in accord with the suggestion that in it also many mutations may be quantitative rather than qualitative changes. As a corollary, the evidence of polyploidy supplies an alternative explanation to that of Brink (1932) for the fact which he cites of translocations (*x*-normals) being the same as *o*-normals in maize but non-viable in *Drosophila*. In the former they may result from crossing-over between "homoeologous" chromosomes and therefore not involve any abnormal process. Further, any duplication or deficiency that might be involved would have less effect in a polyploid.

The possibilities relative to hybrid vigour which are inherent particularly in Goldschmidt's (1927) quantitative theory of the gene, and in Fisher's (1930) genetical theory of natural selection, have special significance for maize and *Sorghum* if they are either primary or secondarily-balanced polyploids. On these theories, in any population in which heterozygotes predominate, the heterozygous state should be the optimum one for many pairs of genes. Obligatory allogamous organisms should therefore exhibit a generalised type of hybrid vigour in addition to a more specific type associated with highly differentiated allelomorphs or interacting non-allelomorphs. Hybrid polyploids have in their immediate diploid ancestor hybrid vigour which is presumably due chiefly to the interaction of a number of such highly differentiated allelomorphs. By chromosome doubling these become polymeric genes (using this term here in a wide sense). An autogamous allopolyloid should retain the specific type of hybrid vigour of its diploid ancestor. An allogamous polyploid should come to have in addition a generalised type of hybrid vigour, depending upon small differences between many allelomorphs. Maize and *Sorghum*, which are respectively largely cross-fertilised and largely self-fertilised, appear to fulfil expectation on this hypothesis, so far as comparative evidence is available. The argument is, of course, distinct from the heterosis and dominant gene theories of hybrid vigour, though it embraces elements of both. If valid, it indicates difficulties, apart from those usually considered, cf. Richey and Sprague (1931), in the way of obtaining fully vigorous homozygous lines of maize and has obvious applications to methods of maize breeding.

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Since the cytological work here described was completed, several more plants of Dakota Amber Sorgo have flowered and been found to be partially asynaptic. We may conclude, since the seed was from a hand-selected plant, that it is a mutant type accidentally picked out, which is analogous to Beadle's (1930) asynaptic maize. From the present point of view, the most interesting feature in the asynaptic form is the frequent formation of multivalents. It has been shown (cf. Darlington, 1932) that there is competition in chromosome pairing. If the asynapsis is due to irregularities in splitting and contraction of the leptotene threads, as it has been shown to be in asynaptic oat and wheat dwarfs (Huskins and Hearne, 1933), then release from competition in pairing with certain segments of their complete homologues would leave parts of chromosomes free to pair with homologous segments of other chromosomes which are only "homoeologous," or incompletely homologous, when considered as a whole. Pairing in normal 10-chromosome *Sorghum* species is analogous to pairing in pure line hexaploid wheat and oats, in which the 42 chromosomes ordinarily form 21 bivalents and multivalents occur only rarely. The asynaptic *Sorghum*, on the other hand, resembles *Zea Mays* \times *Euchlaena perennis* hybrids (Longley, 1924), triploid and pentaploid wheat—*Aegilops* hybrids (Kihara and Nishiyama, 1930), and euploid wheat and oat hybrids (Huskins, 1928, strain 26-54, and unpublished data), in all of which multivalents occur much more commonly than in the parent species.

SUMMARY.

In "diploid" *Sorghum* species, $2n = 20$, 10 bivalents are usually found, but quadrivalents and sexivalents occur occasionally.

In the "tetraploid" *S. halepense*, $2n = 40$, quadrivalents, sexivalents and octavalents sometimes occur.

A fragmentally tetrasomic plant of *S. verticilliflorum* was found which was phenotypically normal.

A strain of Dakota Amber Sorgo was found to be partially asynaptic. Multivalents, however, occur in it with unusual frequency.

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CHROMOSOME DIVISION AND PAIRING IN *FRITILLARIA MELEAGRIS*: THE MECHANISM OF MEIOSIS

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(With Seven Text-figures.)

INTRODUCTION.

NEWTON (1927) noted that the meiotic first metaphase chromosomes in pollen mother cells of *Fritillaria Meleagris* are paired only in the region of the point of attachment, as McClung (1927), Janssens (1924) and others have found in the spermatogenesis of several species of *Mecostethus* and related genera of Orthoptera. Newton and Darlington (1930) and Darlington (1932, p. 98-100) have shown in further detail that at late diplotene and early diakinesis in *Fritillaria Meleagris* and some other species of this genus, chromosome pairing is conditioned by chiasmata which are nearly all confined to a restricted region adjacent to the attachment constriction. In this respect it is less extreme than *Mecostethus grossus*, which has usually only one chiasma, and that immediately adjacent to the attachment.

McClung and Janssens both showed that in *Mecostethus grossus* prophase pairing or synapsis occurs only in the region of the attachment constriction. In *Fritillaria Meleagris*, Newton and Darlington stated that: "The normal parasynapsis of a diploid occurs in the prophase. . . that is to say, homologous chromosomes associate probably throughout their length." Later, Darlington (1932, p. 98) states: "The chromosomes appear to be completely paired at pachytene, but a comparative study shows they are not so closely paired as in the related species *F. imperialis*, which has chiasmata formed regularly throughout its length. Small unpaired loops are constantly found at pachytene." As will be shown, our observations on *F. Meleagris* are not in complete agreement.

Observations of several workers in this laboratory on various plants and animals during 1930-1 seemed to indicate that at all stages of both mitosis and meiosis chromosomes tend to exist in a double or paired thread state (see Huskins *et al.* 1932), and from these and later observations, including those here presented, a simple hypothesis of the

mechanism of meiosis relative to that of mitosis has been developed (Huskins, 1933). This hypothesis grew out of studies stimulated by Darlington's "precocity theory" of meiosis, but differs from it in its primary observational basis and in its development. It agrees in the postulate that pairing occurs in the prophase of meiosis to satisfy an affinity in pairs, which in mitosis is satisfied by the splitting of the chromosomes. If omission of the customary mitotic split is the cause of meiotic pairing, then, conversely, presynaptic splitting should inhibit pairing.

In most organisms metaphase "pairing" appears to be conditioned by chiasma formation during pachytene-diplotene—exceptions have been discussed by Huskins (1932). Metaphase "asynapsis" may therefore be due (1) to real asynapsis, that is, to lack of prophase pairing and consequent inability to form chiasmata, or (2) to failure to form chiasmata after synapsis, possibly, as a corollary of Belling's hypothesis, to the chromomeres being too close together at the time of pairing to permit the occurrence of half twists and the formation of chiasmata between them, or (3) to the chiasmata slipping off the ends of the chromosomes before anaphase (see Moffett, 1932). We initially considered the second of these possibilities the most promising hypothesis for the distal "asynapsis" of *F. Meleagris* in view of Newton and Darlington's metaphase observations of chiasmata still close to the attachment and their statement that normal parasynapsis occurs in the prophase. But since they had not figured stages earlier than late diplotene, the possibility remained that it might segmentally be really asynaptic and that critical evidence for the mitosis-meiosis hypothesis might therefore be obtained from it. A study of it was therefore begun, and coincidentally one on "asynaptic" dwarf oats and wheat, which have most of their chromosomes unpaired at metaphase, was conducted by Huskins and Hearne (1933). This latter study showed that unpaired regions of the chromosomes are split, but owing to gross irregularities in the timing relationships of different chromosomes within a single cell it could not be determined with any degree of certainty whether the splitting preceded and prevented pairing or occurred later. The present study seems more conclusive on this point.

MATERIAL AND METHODS.

Corms of *F. Meleagris* were very kindly supplied by F. Cleveland Morgan, Esq., in April of 1932 and 1933 from his rock garden at Senneville, P.Q. Pollen mother cell smears were fixed in La Cour's 2B and

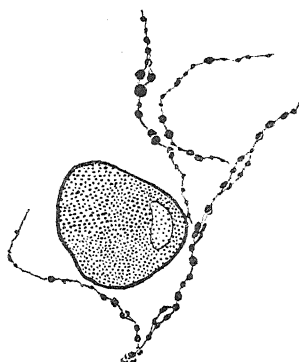


Fig. 1.

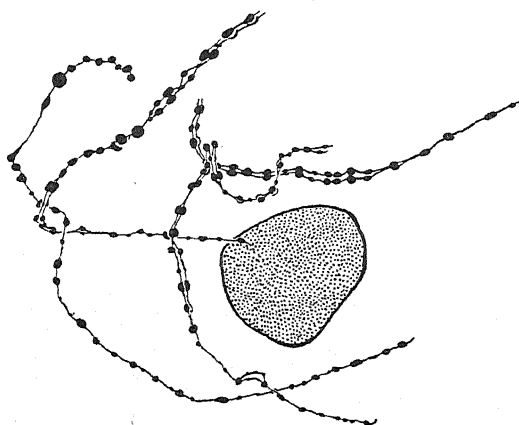


Fig. 2.

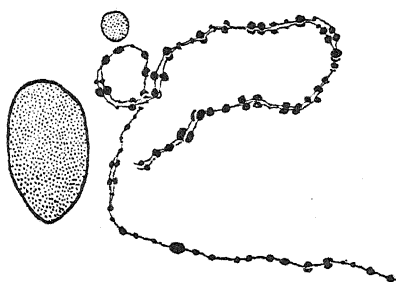


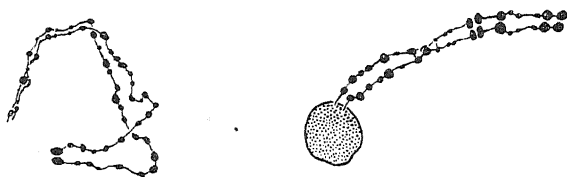
Fig. 3.

Figs. 1-3. Leptotene chromosomes of *F. Meleagris* showing both single and double split regions before the onset of pairing. 2800 \times .

stained in iodine crystal-violet. Drawings were made with the aid of a camera lucida, using a Zeiss 120 \times , 1.3 N.A. objective and 20 \times ocular, which gave a bench-level magnification of 4200 \times . The drawings are reduced to two-thirds in reproduction.

OBSERVATIONS.

Leptotene. At earliest leptotene the chromosomes are found to be in part single and in part split. The general effect, therefore, superficially



Figs. 4-5. Zygotene chromosomes of *Fritillaria Meleagris* showing pairing in unsplit regions of the chromosomes.

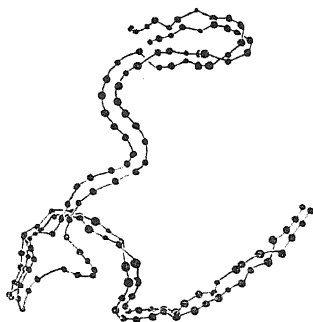


Fig. 6. Zygotene chromosome pair of *Trillium erectum* in which no split is visible before pachytene. Note similarity to Figs. 4 and 5, and differences from Figs. 1-3 which have superficial similarities only. 2800 \times .

resembles zygotene (or amphotene of Janssens). Closer examination shows very definite differences, however, as may be seen from comparison of Figs. 1, 2 and 3 with Figs. 4, 5 and 7. There is in split leptotene threads (Figs. 1, 2 and 3) an absence of the twists commonly found at zygotene and, on the other hand, at zygotene the double nature of the paired chromomeres is rarely or never totally obscured. The spherical chromomeres of the single parts of threads which are elsewhere double are therefore diagnostic of leptotene. It may be stressed that in extended studies of *Trillium* chromosomes which pair throughout their length, we have never observed split chromomeres at leptotene.

Zygotene. At early zygotene, Fig. 4, pairing seems to be initiated either terminally or interstitially and to proceed from paired to adjacent unpaired chromomeres. In the pairing regions of the chromosomes zygotene is normal, as shown in Figs. 4 and 5. Fig. 6 is zygotene of *Trillium erectum* and is inserted for comparison. In the unpaired regions of the chromosomes, split chromomeres are common, as in leptotene. Unfortunately, but not surprisingly, we were unable to trace chromosomes throughout their length at this stage. We cannot therefore say with any degree of certainty which regions of the chromosomes are paired and which split.



Fig. 7. Pachytene chromosome pair of *Fritillaria Meleagris* showing unpaired, split regions at the left and paired, unsplit lengths at the right. The secondary split of late pachytene is occurring in the paired region at the centre. 2800 \times .

Pachytene. Pachytene is normal in the paired sections of the chromosomes, but in the unpaired sections the chromomeres are nearly all split and fairly widely separated, so that these parts again appear superficially like zygotene. In Fig. 7, the secondary split of late pachytene is just occurring in what we take to be the distal section of the rightmost paired half of this chromosome pair. It has not yet begun in the proximal section of this paired half.

Darlington's observations of complete though loose pairing at pachytene were presumably made at a later phase of the pachytene stage than we obtained. The split, unpaired sections of the chromosomes in our preparations of course resemble early pachytene, but their detailed appearance is clearly different and their nature can be definitely established by tracing them to a point where pairing begins. We never found pairing complete throughout the length of any chromosome pair.

Diplotene—Diakinesis—Metaphase—Anaphase. Our diplotene preparations were not very satisfactory, but, so far as could be ascertained, they agree with the condition illustrated in Newton and Darlington's Fig. 2. Very good diakinesis, metaphase and anaphase preparations were obtained, but these stages have been admirably illustrated by Newton and Darlington and are therefore not repeated here. Like them, we found chiasmata strictly localised near the attachment as a rule, but occasionally occurring distally.

General. One of the most striking features of *Fritillaria Meleagris* is the speed with which its pollen mother cell meiosis is completed. From pre-leptotene to first metaphase requires only three to four days. In *Trillium* under comparable temperature conditions about ten weeks are required from leptotene to first metaphase. Spermatogenesis is completed in *Trillium* about four months in advance of the normal flowering period, in *F. Meleagris* only as the buds open.

DISCUSSION.

These early prophase observations answer the question raised by Darlington (1929) and Newton and Darlington (1930) as to whether chiasmata are localised in formation or move later towards the attachment. The former is the case, as reported by Janssens and McClung in *Mecostethus*. It may be noted in passing, that Hearne and Huskins (*in litt.*) have found chiasmata to move towards the attachment in *Melanoplus femur-rubrum*; but conditions of chiasma formation and distribution are quite different in that organism.

The failure of chiasma formation in distal regions of the chromosomes is evidently related to the occurrence of splitting in the unpaired threads, and the occurrence of split chromomeres at leptotene before any pairing has begun seems conclusively to show that it is the splitting which inhibits pairing.

Regarding the time of meiotic chromosome splitting, McClung (1927) writes: "In the last secondary spermatogonial telophase itself, clear evidence of lengthwise chromosome division is afforded. For a long time I was not convinced of this condition, but in the beautifully clear nuclei of *M. gracilis* the separations of the chromatids are so definite as to be unquestionable. At least in this material we must believe that the chromosomes are already equationally divided at the time of synapsis." He figures the telophase split in *Leptysmia marginicollis* and *Pseudopomala brachyptera*. Robertson (1931) and others have made similar observations.

Apparently opposed to these observations are those of numerous cytologists who report the leptotene threads as definitely single. If one accepts both sets of observations, two lines of reconciliation are obvious. First, split chromosomes may re-fuse and become optically single again. Second, conditions may differ in different organisms and it may be possible to place slightly different interpretations on some of the observations. In favour of the first is McClung's observation that in *Mecostethus* the telophasic doubleness is still visible at preleptotene under favourable conditions but that when the fine leptotene thread is spun out no subdivisions can be seen. The complete pachytene pairing recorded by Darlington (1932) in *Fritillaria Meleagris* can also, on the mitosis-meiosis hypothesis, be interpreted to give indirect support to this assumption. We shall, however, attempt to state a case for the second of these alternatives in the light of the present observations.

The time of mitotic chromosome splitting is, of course, also involved in the problem. Kaufmann (1926*a*), Sharp (1929), Hedayetullah (1931) and others have shown that in somatic mitosis the chromosomes split in the division preceding that in which their halves are separated. These observations have been discounted as optical illusions by Darlington (1932), who maintains that splitting occurs during the "resting stage." We have found very clear evidence of the split in the late prophase of the preceding division in both root-tip and pollen-grain mitosis of several plants, and especially clearly in *Trillium erectum* (*in litt.*). Some of the evidence (A. W. S. Hunter, M.Sc. Thesis, McGill) includes end-views of chromosomes and is therefore free from Darlington's objection that doubleness is an optical illusion due to the chromosome being a "hollow cylinder."

Kaufmann (1926*b*) has shown that in the meiotic prophase of *Podophyllum* "Occasionally free ends of intertwined homologues show themselves to be longitudinally divided, the split separating parallel spiral threads. This," he remarks, "is what would be predicted from a consideration of the longitudinally split anaphase and telophase chromosomes of the somatic mitosis."

But it may be stressed that it is in *free* ends of paired chromosomes that the doubleness was observed by Kaufmann, and that it was in *Mecostethus* with its very restricted pairing segment that McClung found the telophase split clear. And somatic telophase splitting need by no means lead to the prediction of a similar condition in meiosis. On the contrary, it is, we consider, in just this regard that a difference might be expected.

Darlington's "precocity theory" of meiosis is based on the generally accepted view that at the earliest stages of the meiotic prophase in which the chromosomes are sufficiently contracted to become visible, they are single; whereas in mitosis they are already double at a comparable stage of contraction. The theory then postulates that at a certain stage of contraction there is a strong affinity between chromosome threads in pairs. In mitosis this is satisfied by the approximation of split halves; in meiosis by the pairing of whole chromosomes.

The present observations strongly support this part of Darlington's hypothesis, and the observations of McClung, Kaufmann, Robertson and others referred to can be interpreted according to it. In their illustrations, meiotic prophase doubleness is all in unpaired segments of the chromosomes, as we find in *Fritillaria Meleagris*. To upset this part of the "precocity theory" evidence of pre-leptotene splitting along the entire length or at least the pairing length of chromosomes with localised pairing, or along considerable sections of chromosomes with random chiasma formation, is needed. The observations of pre-synaptic splitting so far made being almost exclusively on clearly exceptional material tend rather to support the rule.

But if our observations, and those of others referred to, of chromosome splitting occurring in the preceding division are valid, Darlington's assumption of mitotic chromosome splitting occurring in the "resting stage" immediately preceding the division in which separation of the halves occurs is evidently invalid, and therewith the part of his "precocity theory" which postulates that meiosis is initiated by a precocious contraction preceding the normal mitotic resting stage division of the chromosomes breaks down. It has been suggested (Huskins, 1932) that pre-meiotic inhibition of splitting may, instead, initiate meiosis.

For the mechanism of the *procedure* of meiosis relative to mitosis, an hypothesis similar to Darlington's but differing significantly in its observational basis has been formulated. The essence of this "mitosis-meiosis hypothesis" (Huskins, 1933) is that at *all* stages of both mitosis and meiosis chromosome threads or, more properly, chromomeres have an attraction in pairs and a repulsion between pairs of pairs. The observations on which it is based show (1) that mitotic chromosomes are effectively split a full division cycle before the halves are separated; (2) that chromosomes which pair normally throughout their length are single at leptotene and split after pairing, during pachytene; (3) that there is then a repulsion between pairs of paired chromatids during diplo-

tene; (4) that a second split¹ (provisionally termed the "tertiary" split, since the first *split* is commonly called the secondary one to distinguish it from the primary plane of separation between paired homologues) occurs during late meiotic prophase at about the same time or slightly later than that of mitosis; and (5) that the customary prophase split is omitted in the second meiotic division.

McClung (1927) notes that the closely paralleled four-thread pachytene stage is of brief duration and difficult determination in *Mecostethus lineatus*, though it is very marked in *Stenobothrus*. He remarks further that pre-leptotene conditions are very different in the genus *Mecostethus* from "other animals and even other Acrididae." Darlington's (1932) observation that the chromosomes of *Fritillaria Meleagris* are completely paired at pachytene, though not as closely as those of *F. imperialis*, brings it further in line with *Mecostethus lineatus*. This stage, which must occur later than the phase of pachytene which we have illustrated, was not present in our material, nor did Janssens observe it in *M. grossus*. It appears to be analogous to the pachytene pairing of non-homologous chromosomes observed by McClintock (1932) in *Zea Mays*.

SUMMARY.

Fritillaria Meleagris has chiasmata almost exclusively localised in the region of the attachment constriction.

Segments of the chromosomes are already split at leptotene.

At zygotene pairing occurs only along unsplit segments.

During pachytene the secondary split occurs in the paired segments, while the split halves of the unpaired segments become more clearly separated.

The observations are discussed in relation to observations of McClung and others on Orthoptera with localised chromosome pairing and to hypotheses on the mechanism of meiosis.

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¹ This split is observed in the *chromonema*; Darlington's suggestion that the *chromosome* is a hollow cylinder therefore does not apply to it.

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CYTOLOGICAL STUDIES IN COTTON.

II. TWO INTERSPECIFIC HYBRIDS BETWEEN ASIATIC AND NEW WORLD COTTONS.

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(With Thirteen Text-figures.)

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I. INTRODUCTION AND HISTORY.

THE object of the present study is a cytological examination of the species hybrids described by Dr S. C. Harland (Harland, 1932). The main part of the study deals with a cytological comparison of two hybrids between Asiatic and New World cottons. One of these hybrids was slightly fertile and had $2n=39$, while the other hybrid was sterile and had $2n=52$. The chromosome numbers have been counted in a few plants from the first and second back-cross of the fertile hybrid to New World cotton. A closer cytological examination of these and the later generations has been postponed for later study.

Asiatic and New World cottons represent taxonomically the two main groups of all cultivated cottons. The taxonomical separation has been shown by a number of workers (cf. Skovsted, 1933) to be associated with a cytological separation into groups, one with $n=26$ (New World cottons) and the other with $n=13$ (Asiatic cottons). Hybrids between these two groups are therefore of considerable interest both from an economic and a scientific viewpoint.

Several workers have studied the cytology of hybrids between Asiatic and New World cottons:

1. Zhurbin (1930) studied the meiosis in a hybrid, *Gossypium her-*

baceum L. \times *G. hirsutum* L. He found 13 univalent and 13 bivalent chromosomes in the first meiotic division.

2. Nakatomi (1931) studied the meiosis in two different hybrids, *G. hirsutum* L. \times *G. herbaceum* L. and *G. barbadense* L. \times *G. herbaceum* L. He found that 13 univalent and 13 bivalent chromosomes were usually formed in the first meiotic division in the hybrids. The metaphase of the first meiotic division is illustrated with four figures, all of which show 13 univalent chromosomes. His illustrations of the bivalents are not, however, quite so convincing. For instance, Fig. 4 *b* seems to show polyvalents, and the only pollen mother cell which is complete (Fig. 2) looks rather like 13 univalents + 11 bivalents + 1 tetravalent, than like 13 univalents + 13 bivalents as stated in the explanation of the figure. The second meiotic division also showed irregularities, and Nakatomi concluded that the sterility of the hybrids was due to the formation of abortive germ cells.

3. Szymanek and Gavaudan (1932) claimed to have found $n=12$ in a hybrid between *G. herbaceum* L. \times *G. hirsutum* L., and later Szymanek (1932) reported having studied several other hybrids between Asiatic and New World cottons. These two authors stated the chromosome numbers of the pure species in approximate terms but even these are deeply contrasted with the results obtained by other workers (cf. Skovsted, 1933). This can clearly be seen from the following:

Szymanek, 1932	Other workers (cf. Skovsted, 1933)	
	$2n$	$2n$
<i>G. vitifolium</i> var. Ishan	20-26	= <i>G. barbadense</i> L. 52
<i>G. punctatum</i> var. Baroueli	18-22	} = <i>G. purpurascens</i> Poir. 52
" var. Koriba	20-26	
<i>G. arboreum-sanguineum</i>	c. 20	= <i>G. arboreum</i> L. 26

Szymanek explained this disagreement as being due to a variation in the number of chromosomes within the species in accordance with the latitude, according to Hagerup's hypothesis (Hagerup, 1932). The writer, however, would consider a confusion in the taxonomy of the species a much more plausible explanation. The result $n=12$ in a hybrid between Asiatic and New World cottons is therefore left out of further discussion.

4. Longley (1933) described the meiosis in a hybrid between Asiatic and New World cottons. While he stated that the number of paired chromosomes varied, he did not mention the extent of the variation which he found. The first meiotic division is illustrated with only one figure, which shows a metaphase with 11 univalent and 14 bivalent

chromosomes. Evidently Longley did not attribute much importance to the exactness of these numbers, since he did not even mention that they differ from the observations of Nakatomi, whose results he quoted. Longley mentioned that he had difficulties in distinguishing between univalent and bivalent chromosomes. This may be explained by the fact that he studied the metaphase from a polar view which, in the writer's experience, does not give such exact information as a side-view. The results of Zhurbin (*loc. cit.*) and Nakatomi (*loc. cit.*) agree in that 13 is the minimum number for univalent chromosomes found in a hybrid of this kind. Further information seems therefore desirable to show whether there exists some fundamental difference between the hybrids studied by these authors and the one studied by Longley, or whether this is merely the result of the miscount by the latter author of a single pollen mother cell analysed from an unfavourable visual angle.

Only two conclusions seem justified from the above-mentioned studies:

1. A hybrid between New World and Asiatic cottons showed about 26 conjugated and 13 univalent chromosomes in the first meiotic division, but it was impossible to determine whether the conjugation was due to auto- or to allosyndesis.
2. The irregularities in the meiotic division explained the sterility of the hybrid.

II. MATERIAL AND TECHNIQUE.

The two plants studied appeared, as mentioned above, in Dr S. C. Harland's experiments. The fertile hybrid was a result of a cross between *G. barbadense* L. \times *G. arboreum* L., while the sterile hybrid came from a cross made the opposite way, using Asiatic cotton as female, namely (*G. arboreum* L. \times *G. herbaceum* L.) $F_1 \times ((G. hirsutum$ L. \times *G. barbadense* L.) \times *G. barbadense* L.) selfed. The fertile hybrid showed, as expected, $2n=39$, but the sterile hybrid had 52 chromosomes and was more vigorous. The hybrid nature of the latter type is easily seen from some of its morphological characters. Thus, its nectaries outside the bracts are a character from New World cotton, while its petal spot is one characteristic of Asiatic cotton. Its origin indicates that an egg from Asiatic cotton containing a diploid number of chromosomes was fertilised by a normal pollen grain from New World cotton.

The root tips were fixed in Navashin's solution, which gives excellent results when one's aim is the counting of the chromosomes. The con-

strictions of the chromosomes are not as a rule very clear, so that a detailed study of their morphology was not possible. Further experiments are being carried on, however, in an attempt to find a fixative that will be suitable for this purpose. The slides were stained in Gentian Violet according to Johansen's technique (Johansen, 1932 *a*), but the 10 and 30 per cent. alcohols with picric acid, the 95 per cent. alcohol with ammonia, and the absolute alcohol have all been omitted. Excellent results have been obtained in this way, both with different species of *Gossypium*, and with other species of Malvaceae having small chromosomes. It has thus been possible to count quite easily up to 112 somatic chromosomes (in *Pavonia spinifex* (L.) Cav.), so that the writer is much more satisfied with this technique than its inventor (cf. Johansen, 1932 *b*). Most of the slides made more than a year ago have kept excellently despite the tropical climate.

The technique employed for the buds was the same as that described in detail elsewhere (Skovsted, 1933).

III. THE SOMATIC CHROMOSOMES.

The somatic chromosomes of *G. arboreum* have already been described (Skovsted, 1933). Re-examination has confirmed previous conclusions. In recent studies, however, the sizes of chromosomes have consistently been measured and from the accumulated data the interesting fact emerges that whereas the chromosomes of all Asiatic cottons are of equal size and large, the chromosomes of New World cottons are of two sizes, half being large and the other half relatively small. This is seen in favourable cytological material such as haploid *G. barbadense* (Fig. 2, line 3 of Table I).

Chromosome sizes in haploid *G. barbadense*:

13 small	average size	c. 1.25 μ
13 large ¹	„	c. 2.25 μ

Where the chromosome number is large, as in a diploid New World cotton with 52 chromosomes, such distinctions in size may not be so apparent (Fig. 3, line 4 of Table I).

The distinction between these two groups of somatic chromosomes is maintained in the hybrids. Fig. 4 shows a metaphase from the hybrid with 39 chromosomes (measurements in line 5 of Table I). The measurements from another metaphase are shown in line 6 of Table I. Here again the separation between the two groups is clear. Thirteen small

¹ A certain amount of correction has been made for some of the larger chromosomes in the way suggested by Lewitsky (1931).

TABLE I.

*Length of somatic chromosomes in Asiatic and New World cottons and their hybrids measured in mm.
at a magnification of 4700 times¹.*

		1μ															2μ															3μ														
Length in mm. ...		4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15																						
1.	Asiatic cotton, Fig. 1	1	.	2	2	1	1	5	2	3	2	5	1	1																						
2.	Asiatic cotton, (Fig. 1, Skovsted, 1933)	1	2	1	1	5	1	2	2	2	4	1	3	1																							
3.	Haploid New World cotton, Fig. 2	.	1	1	1	2	5	3	.	.	2	1	1	1	2	.	2	3	.	.	.	1																								
4.	Diploid New World cotton, Fig. 3	.	.	1	2	1	7	6	8	1	.	3	4	3	1	3	1	6	2	1	.	1	1																							
5.	Hybrid with 39 chromosomes, Fig. 4	4	6	3	.	.	1	4	1	2	1	6	2	5	1	2	1																													
6.	Hybrid with 39 chromosomes	2	2	6	2	1	.	2	1	2	4	2	5	4	2	1	1	2																												
7.	Hybrid with 52 chromosomes, Fig. 5	.	1	2	3	2	3	2	.	2	2	2	2	3	4	6	3	5	1	.	3	1	1																							
8.	Plant with 58 chromosomes from the second back-cross	3	.	4	4	3	7	4	3	.	2	4	1	6	2	5	4	.	1	.	1	3	1																							

¹ Reading down the table of chromosome-size frequency numbers, it is apparent that Asiatic cottons are represented by a group of 26 large chromosomes—for convenience these have been bracketed together. On the other hand, half of the chromosomes of New World cottons are definitely of smaller size and half of larger size corresponding to the Asiatic group. See text.

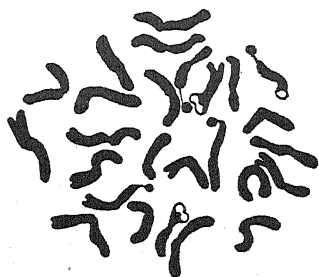


Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

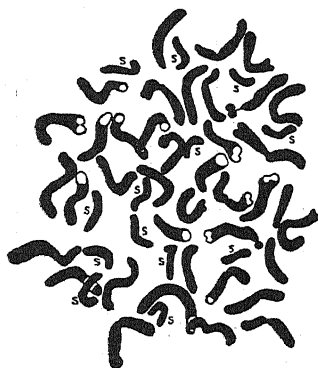


Fig. 5.

Figs. 1-5. The somatic chromosomes of Asiatic and New World cottons and of their hybrids ($\times 3500$).

Fig. 1. Asiatic cotton, *G. arboreum* ($2n=26$), 4 chromosomes with satellites.

Fig. 2. Haploid New World cotton, *G. barbadense* ($2n=26$), with 13 small (*s*) and 13 larger chromosomes.

Fig. 3. Diploid New World cotton, *G. barbadense* ($2n=52$), with 26 small (*s*) and 26 larger chromosomes.

Fig. 4. Hybrid, *G. barbadense* \times *G. arboreum*, with 39 chromosomes, 13 small (*s*) and 26 larger ones.

Fig. 5. Hybrid (*G. arboreum* \times *G. herbaceum*) $F_1 \times$ (*G. hirsutum* \times *G. barbadense*) progeny, with 52 chromosomes, 13 small (*s*), and 39 larger ones.

chromosomes, presumably from New World cotton, form one group, while the other group comprises 13 chromosomes from New World cotton and 13 from Asiatic cotton.

The somatic chromosomes of the hybrid with 52 chromosomes are shown in Fig. 5 and the measurements in line 7 of Table I. The two groups are here made up of 13 small chromosomes, and 39 larger ones. This confirms the view that an egg of Asiatic cotton containing 26 chromosomes was fertilised by a normal pollen grain from New World cotton. The group of small chromosomes is therefore entirely from New World cotton, whereas the other group includes 13 chromosomes from New World cotton and 26 from Asiatic cotton.

The chromosome numbers have been counted in five plants from the first back-cross of the fertile hybrid to New World cotton. The following numbers were found: $2n=53$, $2n=63$, $2n=63+1$ fragment and $2n=65$.

When twelve plants were examined from the second back-cross between New World cotton and the above-mentioned plant with 65 chromosomes, the following numbers were found: $2n=55$ (54?), $2n=56$, $2n=58$, $2n=59$, $2n=c. 60$, $2n=61$, $2n=c. 62$, $2n=62+1$ fragment, $2n=c. 63$, $2n=63$ and $2n=c. 64$, indicating that the functional pollen grains contained between 26 and 39 chromosomes. The measurements of the chromosome complement of one of these plants are shown in line 8 of Table I.

IV. THE MEIOSIS OF THE HYBRID WITH 39 CHROMOSOMES.

This hybrid in its earlier stages of meiosis resembles the triploid Asiatic cotton to such an extent, that only reference to the description of this plant (Skovsted, 1933) will be made here.

As mentioned above, previous workers on similar crosses have apparently not noticed any higher chromosome configurations than bivalents. The writer, however, found that tri-, tetra-, penta- and hexavalents are also formed (Figs. 6-8 and Table II). For this analysis only side-views of the metaphases have been used. The totals are given in groups of 20 pollen mother cells to enable comparison with the results obtained from the triploid Asiatic cotton and from the hybrid with 52 chromosomes. Moreover, a comparison of the two sets of totals shows that a study of 20 pollen mother cells gives a fairly good idea of the conjugation of the chromosomes. Zhurbin (*loc. cit.*) and Nakatomi (*loc. cit.*) found 13 univalents+13 bivalents, a type which does not correspond to any of the 40 pollen mother cells analysed by the writer.

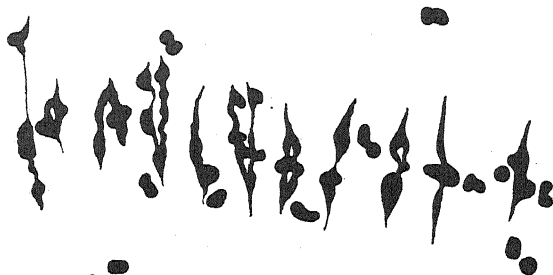


Fig. 6.

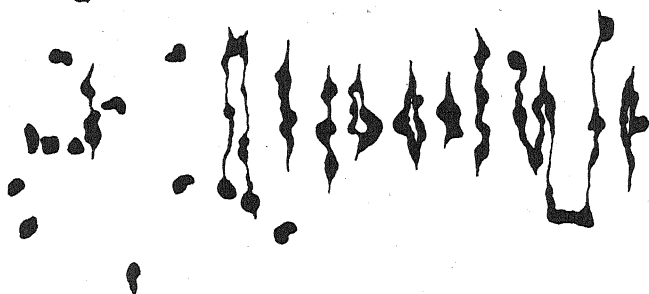


Fig. 7.



Fig. 8.

Figs. 6-8. Side-views of metaphases from the first meiotic division in the hybrid with 39 chromosomes ($\times 3100$).

Fig. 6. Showing 13 univalents, 7 bivalents and 4 trivalents (from left to right: 1 III, 1 II, 1 III, 3 I, 1 III, 1 I, 1 II, 1 I, 1 III, 1 II, 1 I, 1 II, 1 I, 1 II, 2 I, 1 II, 3 I).

Fig. 7. Showing 13 univalents, 8 bivalents, 1 tetravalent and 1 hexavalent (from left to right: 6 I, 1 II, 6 I, 1 IV, 1 I, 6 II, 1 VI, 1 II).

Fig. 8. Showing 14 univalents, 10 bivalents and 1 pentavalent (from left to right: 4 I, 1 V, 1 I, 2 II, 1 I, 1 II, 1 I, 6 II, 1 I, 1 II, 6 I).

Hexavalents were the highest chromosome configurations observed in this hybrid. Some of the polyvalents figured above are forming rings

TABLE II.

Showing chromosome conjugation in 40 pollen mother cells in the hybrid with 39 chromosomes.

Pollen mother cell	Uni-valent	Bi-valent	Tri-valent	Tetra-valent	Penta-valent	Hexa-valent
1	13	10	2	.	.	.
2	14	9	1	1	.	.
3	13	10	2	.	.	.
4	13	11	.	1	.	.
5	17	7	.	2	.	.
6	13	9	.	2	.	.
7	14	10	.	.	1	.
8	17	9	.	1	.	.
9	14	11	1	.	.	.
10	14	6	3	1	.	.
11	17	8	2	.	.	.
12	13	8	.	1	.	1
13	13	8	2	1	.	.
14	17	6	2	1	.	.
15	14	8	.	1	1	.
16	20	7	.	.	1	.
17	13	7	1	1	1	.
18	13	7	4	.	.	.
19	17	8	2	.	.	.
20	14	7	2	.	1	.
21	13	8	2	1	.	.
22	13	10	.	.	.	1
23	15	9	2	.	.	.
24	14	9	1	1	.	.
25	13	8	.	1	.	1
26	14	7	2	.	1	.
27	13	8	.	1	.	1
28	15	9	2	.	.	.
29	15	10	.	1	.	.
30	13	8	.	1	.	1
31	14	11	1	.	.	.
32	13	6	3	.	1	.
33	14	9	1	1	.	.
34	13	7	1	1	1	.
35	13	8	2	1	.	.
36	14	10	.	.	1	.
37	13	8	.	1	.	1
38	19	10
39	16	9	.	.	1	.
40	17	7	.	2	.	.
Total (1-20)	293	166	24	13	5	1
Total (21-40)	284	171	17	12	5	5

and may indicate segmental interchange, although it is also possible to explain these on a polyploid basis. Pseudo-polyvalents, probably the result of interlocking, were found in a few cases, Fig. 9, a configuration

formed of a tetra- and a pentavalent. Fig. 10 shows two interlocking trivalents.

It is a characteristic of all the pollen mother cells examined that 13 is the lowest number of univalent chromosomes. This is not only the case with the 40 pollen mother cells shown in Table II, but has also been observed in a number of cells where it was impossible to analyse all the conjugated chromosomes. During the metaphase of the first meiotic division, the univalents are scattered throughout the protoplasm near the poles and between the conjugated chromosomes. The shape of some of the univalents indicates that they are attached to spindle fibres, and that they sometimes split together with or even before the separation of the conjugated chromosomes.



Fig. 9.



Fig. 10.

Figs. 9-10. Interlocking of polyvalents from the hybrid with 39 chromosomes ($\times 3100$).

Fig. 9. A tetraivalent and a pentavalent.

Fig. 10. Two trivalents.

Both the anaphase of the first meiotic division and the second meiotic division show a number of irregularities, many of which are rather similar to those described from *Oenothera* by Gates and Thomas (1914). Unreduced gametes seem to be formed by fusion of nuclei in the second meiotic division.

The result of the meiosis is found as monads, dyads, triads, tetrads and pentads, and the resulting pollen grains varying in size from small ones to others considerably larger than those of New World cotton. Only about 30 per cent. of the pollen grains contain protoplasm and these are all included in the larger forms, while the smallest ones are all quite empty.

V. THE MEIOSIS OF THE HYBRID WITH 52 CHROMOSOMES.

A study of the conjugation of the chromosomes in this hybrid helps to solve the difficulties encountered in the hybrid with 39 chromosomes, i.e. whether the chromosome conjugation in that plant was due to auto-

or to allosyndesis. Similar technical methods were employed in this investigation. Although the chromosome number made this study more difficult, it was still possible to select 20 sufficiently clear pollen mother cells to permit of analysis (Table III, Figs. 11-13).

From Table III it will be seen that the minimum number of univalents in this hybrid is thirteen. Twenty pollen mother cells are found to have totals of 293 and 284 univalents in the hybrid with 39 chromosomes, and a total of 315 in the hybrid with 52 chromosomes, numbers which agree well.

TABLE III.

Showing chromosome conjugation in 20 pollen mother cells in the hybrid with 52 chromosomes.

Pollen mother cell	Univalent	Bi-valent	Tri-valent	Tetra-valent	Penta-valent	Hexa-valent
1	19	9	5	.	.	.
2	14	6	5	.	1	1
3	13	4	6	2	1	.
4	17	7	5	.	.	1
5	16	5	6	2	.	.
6	20	6	5	.	1	.
7	18	4	6	2	.	.
8	14	3	5	3	1	.
9	16	6	5	1	1	.
10	17	7	7	.	.	.
11	14	1	9	1	1	.
12	14	7	4	3	.	.
13	13	9	4	1	1	.
14	16	7	6	1	.	.
15	13	4	9	1	.	.
16	13	7	7	1	.	.
17	17	9	4	.	1	.
18	14	5	8	1	.	.
19	15	6	7	1	.	.
20	22	6	6	.	.	.
Total	315	118	119	20	8	2

Observations of the anaphase and the second meiotic division in this plant resemble closely those already described for the hybrid with 39 chromosomes. Only one difference has been encountered during these stages, and that is that fusion of the nuclei has not been observed, and gametes containing the unreduced number of chromosomes are therefore not formed.

Variations in the size of the pollen grains were similar to those in the hybrid with 39 chromosomes, the percentage of protoplasm-filled pollen grains being about 27, which shows that the percentage of "good pollen" is approximately the same in the fertile and in the sterile hybrid.

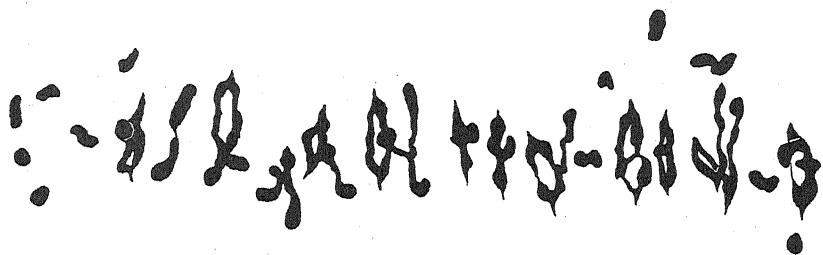


Fig. 11.

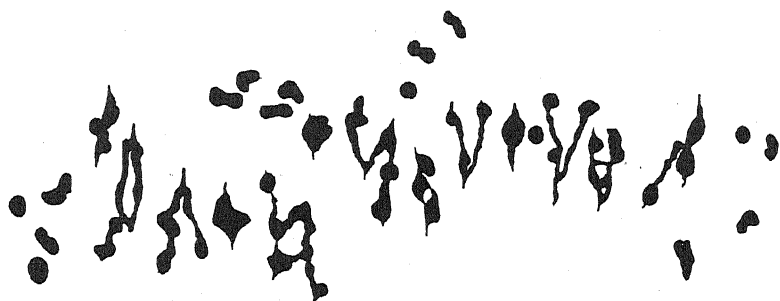


Fig. 12.

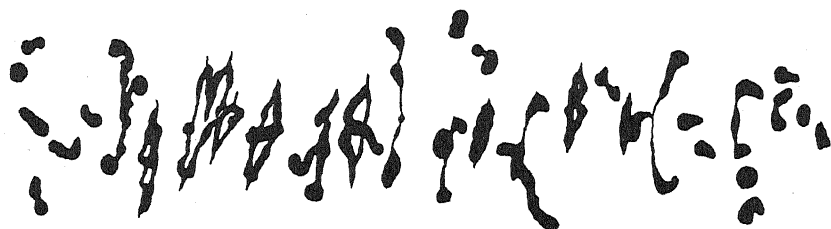


Fig. 13.

Figs. 11-13. Side-views of metaphases from the first meiotic division in the hybrid with 52 chromosomes ($\times 3100$).

Fig. 11. Showing 13 univalents, 4 bivalents, 6 trivalents, 2 tetravalents and 1 pentavalent (from left to right: 6 I, 1 III, 1 II, 1 IV, 2 III, 1 IV, 2 II, 1 III, 2 I, 1 III, 1 I, 1 II, 2 I, 1 V, 2 I, 1 III).

Fig. 12. Showing 16 univalents, 5 bivalents, 6 trivalents and 2 tetravalents (from left to right: 4 I, 1 II, 2 III, 1 II, 4 I, 1 IV, 1 II, 1 IV, 1 II, 3 I, 1 III, 1 II, 1 I, 3 III, 4 I).

Fig. 13. Showing 17 univalents, 7 bivalents, 5 trivalents and 1 hexavalent (from left to right: 6 I, 1 III, 1 II, 1 VI, 1 II, 2 III, 3 II, 2 I, 1 III, 1 II, 1 I, 1 III, 2 I, 1 II, 6 I).

VI. GENERAL DISCUSSION.

From a study of somatic chromosomes, it is evident that New World cottons may be divided into two groups on the basis of chromosome size, half being definitely smaller than the others, a feature absent in Asiatic cottons. In interspecific hybrids between Asiatic and New World cottons, the separation of the two groups of somatic chromosomes in New World cotton is presumably maintained, but as a matter of fact, it is not possible to distinguish between the New World chromosomes and those of the Asiatic cotton. Thus the hybrid with 39 chromosomes contains 13 small and 26 larger chromosomes; and the hybrid with 52 chromosomes contains 13 small and 39 larger chromosomes.

The chromosomes of the hybrid with 39 chromosomes differ considerably in size from those of the parents and the hybrid with 52 chromosomes. In one root, however, chromosomes of similar size were found in the hybrid with 52 chromosomes, all the other roots examined containing chromosomes of the variable sizes shown in line 7 of Table I. This suggests that variation in size is not the result of a genetic factor, but is probably due to some physico-chemical reaction. It is interesting to note that the shortening of the chromosomes does not affect the separation of the two groups. This indicates that the important question put forward by Lewitsky (1931), i.e. "whether the length of the chromosomes of a given set changes proportionally equally, or not," may here be answered affirmatively.

It has been seen that it is impossible to distinguish the somatic chromosomes of Asiatic cotton from the set of larger chromosomes in New World cotton on the basis of size alone. On the other hand the morphology of the chromosomes reveals a difference in the number of satellites. Thus the hybrid with 39 chromosomes shows three satellites, two of which have come with the 13 chromosomes from Asiatic cotton, while only one chromosome in the set of 26 in New World cotton carries a satellite. The writer agrees with Lewitsky (*loc. cit.*) that the loss of a satellite in a chromosome is probably not a change of a very fundamental nature.

The study of the somatic chromosomes lends support to the view that the hybrid with 52 chromosomes probably arose from the fertilisation of a diploid egg of Asiatic cotton by a normal pollen grain from New World cotton. In the present state of knowledge it seems reasonable to infer, provisionally, that the diploid egg was formed as a result of a duplication which occurred after the reduction division. The genetical

observations supporting this were as follows: the mother plant is known to be heterozygous for red corolla (**R**)—a dominant character (Hutchinson, 1932) but one not phenotypically present in the hybrid; it is, however, visible in the hybrid with 39 chromosomes and in the back-cross plant with 65 chromosomes, which probably comprises two haploid sets of chromosomes from New World cotton, and one haploid set from Asiatic cotton; this indicates that one or two sets of chromosomes from New World cotton do not affect the dominance of the gene for red, (**R**) from Asiatic, though as Harland (*loc. cit.*) has pointed out, the phenotypic expression is weakened. It seems, therefore, less convincing to assume that an extra set of chromosomes from Asiatic cotton added to a hybrid with 39 chromosomes should change the dominance of the gene, than to adopt the explanation of a duplication which took place after the reduction division.

Considerable variation has been observed in the conjugation of the chromosomes in the two hybrids during the first meiotic division. It is, however, a characteristic of all the pollen mother cells examined that 13 is the lowest number of univalent chromosomes found in either hybrid. This proves that *the chromosomes of Asiatic cotton are homologous with half the number of chromosomes in New World cotton, while the remaining half are left out as univalents*. The conjugated chromosomes are found in configurations from bivalents to hexavalents. Bivalents were the highest chromosome configurations which were noticed by Zhurbin (*loc. cit.*) and Nakatomi (*loc. cit.*) in their studies of similar hybrids, despite the fact that Nakatomi's illustrations seem to indicate the presence of polyvalents. The results obtained from triploid Asiatic cotton together with the data obtained from the present study suggest that polysomes will be formed in any hybrid between Asiatic and New World cottons—a view supported by the writer's observations on another hybrid of this type¹. The reason why polysomes were overlooked by previous workers is probably attributable to the influence which Rosenberg's classical study of *Drosera longifolia* × *D. rotundifolia* (Rosenberg, 1909) exercised over a number of cytological studies of species hybrids. Also, as Winge (1917) has pointed out with regard to chromosome numbers, there is "a general inclination in the human mind, when dealing with numerical questions, to grasp at the 'nice' figures." The hybrid between Asiatic

¹ This hybrid came to the station some years ago from Dr A. E. Longley in Washington. Two pollen mother cells had the following chromosome complements: 13 univalents, 8 bivalents, 1 tetravalent and 1 hexavalent; and 14 univalents, 8 bivalents, 1 trivalent and 1 hexavalent.

and New World cottons are similar types to the *Drosera* hybrid which showed 10 univalents and 10 bivalents. Rosenberg explained the conjugation of the chromosomes as the result of allosyndesis, while Meurman (1928) and Darlington (1931) are emphatic that it yet remains an open question whether the chromosome conjugation is due to auto- or allosyndesis. The hybrids here studied leave us in no doubt on that point since there are at least 13 univalents in either hybrid.

The total of the different chromosome configurations found in 20 pollen mother cells is used in Table IV as a symbol for the conjugation of the chromosomes. The first two lines of this table show how accurate an estimate is obtained by this method. On comparing the two hybrids it is found that the total number of univalents is approximately the same, and the only difference of any significance lies in the number of

TABLE IV.

Showing the totals of chromosome configurations in the two hybrids and in triploid Asiatic cotton (Skovsted, 1933).

Chromosome configuration		Uni-valent	Bi-valent	Tri-valent	Tetra-valent	Penta-valent	Hexa-valent	Septa-valent
New World-Asiatic hybrid	P.M.C. 1-20	293	166	24	13	5	1	.
(39 chromosomes)	P.M.C. 21-40	284	171	17	12	5	5	.
Asiatic-New World hybrid	P.M.C. 1-20	315	118	119	20	8	2	.
(52 chromosomes)								
Triploid Asiatic cotton	P.M.C. 1-20	50	114	120	22	7	2	1
(39 chromosomes)								

bivalents and trivalents. Again, comparison of the hybrid with 52 chromosomes and the triploid Asiatic cotton with 39 chromosomes reveals a striking similarity. There are 260 chromosomes more in 20 pollen mother cells of the hybrid than in the triploid. The whole of this difference lies in the number of univalents, and if disregarded, conformity between the totals of the hybrid with 52 chromosomes and the triploid Asiatic cotton is just as good as between the two sets of 20 pollen mother cells in the hybrid with 39 chromosomes. *This shows that a hybrid comprising 26 chromosomes from New World and 26 from Asiatic cotton exhibits the same type of chromosome conjugation as a triploid Asiatic cotton to which has been added an extra set of 13 univalent chromosomes.*

New World cottons with $n=26$ are known to be tetraploids compared with Asiatic cottons having $n=13$, but so far no closer relationship between these, the two main groups of all cultivated cottons, has been discovered. The present study shows that there are two different sets of chromosomes in New World cotton, one homologous with the chromosomes in Asiatic cotton, and the other entirely different from both this first set and the set in Asiatic cotton. *Evidently New World cottons are*

amphidiploid species formed by the doubling of the number of chromosomes in a hybrid between two species with the same chromosome number but with non-homologous chromosomes (vide Winge's theory of the formation of species, Winge, 1917 and 1932). One of the parental types must then be an Asiatic cotton or a cytologically similar species, while the other species is unknown.

The study of somatic chromosomes reveals the set of larger chromosomes in New World cotton to be of the same size as the chromosome set in Asiatic cotton. When this result is compared with that obtained from a study of the meiosis, it seems reasonable to suppose that the larger chromosomes in New World cotton are homologous with the chromosomes in Asiatic cotton. These conditions appear somewhat similar to those existing in the amphidiploid *Aesculus carnea*, where the difference in the size of the meiotic chromosomes of the two parental species enables a separation to be made in the hybrid also (Skovsted, 1929).

All species of *Gossypium* with $n=13$ which so far have been examined may be divided into two definite groups according to the sizes of their somatic chromosomes: one group has larger chromosomes and includes the species from Asia, Africa and Australia: *G. arboreum* L., *G. herbaceum* L., *G. Stocksii* M. Mast., *G. africanum* Watt, and *G. Sturtii* F. v. M.; the other group, with small chromosomes, contains the North American species: *G. Davidsonii* Kellogg, *G. Harknessii* Brandg., *G. lanceoforme* Miers (= *Thurberia thespesioides* A. Gray) and *G. aridum* comb.nov. (= *Erioxylum aridum* Rose and Standley). Assuming therefore the correctness of the writer's hypothesis, the unknown parent of New World cotton is to be found among the species of this latter group, provided, of course, it has not died out. A cytological study of interspecific hybrids of the genus *Gossypium* is being undertaken to test this possibility, and already crosses have been made in the hope that a synthetic New World cotton may be obtained in the same way as Müntzing (1932) produced the synthetic *Galeopsis tetrahit*.

Presumably the origin of New World cottons goes back to a time when the areas of distribution of the two constituent prototypes overlapped. The changes which have taken place in the chromosomes since that time must be exceedingly small, seeing that the homology between the chromosomes yet remains so good despite the long period of separation. This agrees with observations from Asiatic cottons where the changes during the formation of the two species have not been sufficiently great to disturb the conjugation of the chromosomes to any extent (Skovsted, 1933).

VII. SUMMARY.

1. Of two interspecific hybrids between Asiatic and New World cottons studied, one had $2n=39$ and the other $2n=52$. For the latter, the inference is that a diploid egg from Asiatic cotton had functioned.

2. In a study of the somatic chromosomes of New World cotton it has been found that half of the chromosomes are small and the other half larger, the latter being comparable in size to the chromosomes of Asiatic cotton. The small chromosomes of New World cotton are of the same size as those in diploid wild species from North America. Species from the Old World and from Australia are all characterised by the larger size of their chromosomes.

3. In the first meiotic division it was seen that (1) at least 13 univalent chromosomes are present in both hybrids, and (2) the hybrid with 52 chromosomes shows the same chromosome conjugation as in a triploid Asiatic cotton, but with the addition of an extra set of 13 non-homologous chromosomes.

4. The conclusion was drawn that New World cottons are allopolyploid species. It is thought that these probably originated from a cross between two species of *Gossypium* both with $n=13$ but possessing morphologically dissimilar and non-homologous sets of chromosomes. The inference is that one of the parental species was an Asiatic cotton or a very closely allied type, while the other was probably a New World species characterised by its smaller chromosomes.

Acknowledgment. I wish to express my indebtedness to Dr S. C. Harland, who kindly provided me with the material and with information regarding the types studied, and to Prof. R. Ruggles Gates, F.R.S., who kindly read and criticised the manuscript before publication.

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THE GENETICS OF *NEUROSPORA*.

V. SELF-STERILE BISEXUAL HETEROKARYONS.

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INTRODUCTION.

It has been established in the case of *Neurospora* by Dr B. O. Dodge and verified by the writer, among others, that the formation of perithecia occurs if two mycelia mated together carry nuclei of certain genetic constitutions. A pair of allelomorphs by which the two mycelia must differ has been located in a particular chromosome and their linkage relations studied (Lindegren, 1933). Perithecium formation occurs only if the gametes are such that the zygote will be heterozygous for this pair of allelomorphs. However, exceptional cases have been reported (Lindegren, 1932 *a*) in which the two types of mycelia, (+) and (−), were mated together, but failed to produce zygotes, apparently because of modifying or "sterility" factors.

Moreau and Moreau (1930) have presented cytological evidence which they consider proves that the perithecium is produced parthenogenetically. Moreau and Moruzi (1931) have performed experiments which they claim corroborate this view. Certain of the cytological findings of Moreau and Moreau are inconsistent with the genetical data presented by Dodge (1930) and the writer. These cytological findings are not necessarily incorrect, but rather they are incomplete, since the entire cycle of development of the two anastomosing mycelia was not followed, but merely some of the stages in the formation of the perithecium. The existence of a fusion of, or association of, nuclei at some stage in the cycle was thus not excluded. Their reported finding that the ascus is derived from a mononucleate cell is definitely shown to be incorrect by the genetical situation. As pointed out by Dodge (1932 *b*) and by the writer in his unpublished thesis, this supposition would require a double fusion in *Neurospora*, which the writer has shown by genetical experiments does not occur. The small size of the nuclei leaves it possible that this so-called "mononucleate" cell, from which Moreau and Moreau say the ascus is formed, is really a dikaryotic cell; but the two nuclei are so closely paired that it is not possible to resolve them. This would be consistent with the genetical findings.

Moreau and Moruzi have described experiments which show clearly that perithecia containing ascospores can be produced by a self-sterile mycelium of *Neurospora* through the diffusion of a hormone from a second mycelium into the substrate carrying this self-sterile mycelium. The writer is convinced, by experiments which will be reported, that the self-sterile mycelium which Moreau and Moruzi were able to render self-fertile by means of a hormone is in reality a bisexual mycelium, and that the hormone rendered inactive the genetical complex which was responsible for the sterility.

EXPERIMENTAL.

Dr B. O. Dodge kindly sent the writer four mycelia of *N. crassa* collected directly from nature in Cuba, Mexico and Panama by Dr Weston, and a fifth strain collected directly from nature by Dr Yamanuchi in Japan. One of these mycelia (strain 1) was mated to both sexes of the pure-bred mutant stock of *fluffy*. Both matings were fertile and produced perithecia containing ascospores. In view of this surprising result the five strains were mated with each other in all combinations.

The result for each of the ten combinations is indicated by *P* for the formation of perithecia or by *N* (no perithecia) for sterility, as follows:

1 × 2	1 × 3	1 × 4	1 × 5	2 × 3	2 × 4	2 × 5	3 × 4	3 × 5	4 × 5
<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>

The above results are clearly inconsistent with the idea that each strain is homokaryotic. On this assumption, strain 1 would have to be considered of opposite sex to strains 2, 3, 4 and 5. That would mean that strains 2, 3, 4 and 5 were all of the same sex. But strains 2 and 3 both produce perithecia when mated to either strains 4 or 5.

Each mycelium of the inbred stocks of *N. crassa* described by the writer (1933) was grown from a single ascospore obtained from an ascus containing the full number of eight ascospores. Such a mycelium is homokaryotic and carries only (+) or (−) nuclei. This is not the result when asci with less than eight ascospores are used. Then one might grow a mycelium from a single ascospore which carried both (+) and (−) nuclei. Such a mycelium might produce perithecia when grown alone. Over a thousand matings have been made among the homokaryotic stocks and their progeny in the course of the last five years, and no significant exceptions to fixed unisexuality have been encountered. The fact that perithecia are not produced by a given mating does not necessarily mean that the two mycelia are of the same sex. Sometimes modified genetical constitution (genetical sterility), or unfavourable cultural conditions, prevents two mycelia of different sex from

producing perithecia. One can say that two homokaryotic mycelia are of different sex if they are mated together and produce perithecia. Irregularities involving the failure of perithecia formation are not indicative of sexual identity; irregularities due to the unexpected production of perithecia, such as those occurring when the above five strains were mated, are decidedly significant of the involvement of two sexes. Such a case might be easily mistaken for multipolar sexuality, if one made the false assumption that these strains were homokaryotic.

Seventy-nine matings were made between the five strains which had been collected from nature and various stable inbred stocks. Strains 4 and 5 reacted regularly as simple (-) strains in thirty-four tests. The remaining forty-five tests were of strains 1, 2 and 3, with various homokaryotic stocks whose sex was definitely known. The fertile or perithecia-forming combinations and the sterile or no-perithecia combinations are indicated by *P* and *N* below:

$1 \times (+)$	$1 \times (-)$	$2 \times (+)$	$2 \times (-)$	$3 \times (+)$	$3 \times (-)$
<i>P</i> <i>N</i>	<i>P</i> <i>N</i>	<i>P</i> <i>N</i>	<i>P</i> <i>N</i>	<i>P</i> <i>N</i>	<i>P</i> <i>N</i>
7 2	6* 2	1* 5	8 0	1* 5	7 1

In the above tests there occurred three significant exceptions (*) which prove that strains 1, 2 and 3 had potentialities of more than one type of sex reaction, the bisexuality indicating dikaryotic strains. The sterile matings where fertility could have been expected were more frequent than usual. These failures may be due to the fact that although these strains are dikaryotic, the nuclei are easily separated by somatic segregation, with the result that a particular transfer mated with the tester strain is often homokaryotic and unisexual.

Analyses were made of fifty-four asci from crosses between these strains and the two sexes of the clear-cut, easily classifiable mutant *fluffy*. Usually more than one ascus from each perithecium was dissected; the asci from a single perithecium being enclosed in a bracket in the tabulation below:

$2 \times F(+)$	f f f f	3, 2, 2, 2, 1, 1, 1	$1 \times F(-)$	F F f f 1
"	{ FM fM fm Fm	1	"	f F f — 1
"	{ fm fm FM FM	1	$3 \times F(+)$	{ f f F F 1
"	{ FM fM Fm fm	1	"	{ f F f F 1
"	{ fm Fm FM fM	1	"	{ F f f F 1
"	{ fm FM FM fm	1	"	{ F F f f 1
"	{ fM FM — —	1	"	{ F f f F 1
"	{ f f f f	1	"	{ f f F F 2
"	{ FM fM Fm Fm	1	"	{ F F f f 1
"	{ f f f f	2	"	{ F f f F 1
$1 \times F(+)$	f f f f	4, 1	$5 \times F(+)$	{ F f f F 1
"	{ fD fD fd fd	1	"	{ f f F F 1
"	{ fd fd fD fD	1	"	{ f F f F 1
			"	{ f F f F 1

In the above tabulation, the small letter (*f*) indicates that both ascospores of the respective pair of (1 and 2, 3 and 4, 5 and 6, and 7 and 8) were wild-type or *non-fluffy*. The capital letter (*F*) indicates that the ascospores of the pair were *fluffy*. The number following the description of the asci indicates the number of asci of this kind found in a single perithecium. The upper part of the first column shows the results of the cross or strain 2 by *fluffy* (+). Fifteen asci contained only wild-type ascospores. Seven asci carried four *non-fluffy* and four *fluffy* ascospores. In all the asci carrying *fluffy*, and only in these, there was detected a second character, *melanistic*. The *melanistic* character (*M*) is expressed by the production of an intensely black colour in the substrate in contrast to the colourless substrate of the cultures of wild-type mycelia.

These facts can be explained as follows: The wild-type self-sterile strain 2 is really a heterokaryotic mycelium containing both (+) and (−) nuclei. It does not produce perithecia, because both the (+) and (−) nuclei carry factors which prevent this. But perithecia are produced when strain 2 is grown together with either a *fluffy* (+) or a *fluffy* (−) mycelium. These perithecia result from hybridisation of the *fluffy* (+) nuclei and the (−) nuclei of strain 2, or *vice versa*. The resulting asci produce four *fluffy* and four *non-fluffy* ascospores. But also when mycelium 2 and the *fluffy* (+) or (−) mycelium mingle and anastomose with each other, the factors in the (+) and (−) nuclei of strain 2, which had hitherto prevented them from interacting sexually, are rendered inactive, and zygotes are formed between the (+) and (−) nuclei of strain 2 itself. These zygotes produce eight wild-type ascospores.

An experiment of Moreau and Moruzi (1931) suggests that diffusible hormones are responsible for the release of the inhibition to sexual reactions in the bisexual self-sterile strains. It is possible that the mingling of the cytoplasm of each fertile *fluffy* strain with that of the self-sterile strain 2, following anastomosis, also cancels the effect of the sterility factors. Two perithecia contained both types of asci. This means that these perithecia were initiated by at least two pairs of nuclei, namely one 2 (+) nucleus paired with a 2 (−) nucleus and one 2 (−) nucleus paired with a *fluffy* (+) nucleus. It has previously been shown (Lindegren, 1934) that such perithecia are unusual in certain crosses. In fact, the writer has stated the general rule, based on good genetical evidence, that each perithecium is typically initiated by a single pair of nuclei which subsequently divide conjugately, and whose descendants fuse in each young ascus. In this case all the ascus zygote nuclei produced in a single perithecium are genetically identical. The apparent

contradiction to this rule may perhaps be explained by the fact that the *tan* crosses, by analysis of whose progeny this rule was discovered, produced extremely few conidia, while mycelium 2 produces an abundance of conidia. It is possible that some of the fertile perithecia from the crosses with 2 were produced by conidiation (Dodge, 1932 *a*), while the fertile perithecia in the case of the *tan* crosses were produced by some other means.

The *melanistic* character was very easy to classify. It was expressed only in the asci formed from a $2(-) \times F(+)$ zygote. It was not expressed in any asci formed from a $2(-) \times 2(+)$ zygote. The simplest, but not the only explanation is as follows: The $2(-)$ nuclei carry the gene *melanistic*. The $2(+)$ nuclei carry one or more suppressors of *melanistic* which prevent its expression in any of the products of reduction of the $2(-) \times 2(+)$ zygotes. But the $F(+)$ nuclei do not carry the suppressor, and each $2(-) \times F(+)$ zygote produces four *melanistic* and four *non-melanistic* ascospores. This explanation is consistent with the view already presented (Lindgren, 1933) that mutants are easily obtained in *N. crassa* by inbreeding, with the consequent segregation of mutant genes from suppressors. A critical study has been planned with the object of discovering if a *melanistic* suppressor exists in *N. crassa* similar to those described by Bridges (1932) in *Drosophila*.

Asci from three perithecia from the $1 \times F(+)$ mating were analysed. Presumably they were formed by $1(-)$ nuclei pairing with $1(+)$ nuclei under the stimulus of hormones or cytoplasm from the $F(+)$ mycelium. One perithecium contained two asci heterozygous for the mutant *downy*. All four asci from one perithecium either did not carry the gene *downy*, or carried *downy* suppressors. In either case strain 2 is shown to be a heterokaryon containing at least three kinds of nuclei. One possibility, on the basis of the first assumption, would be as follows: $d(+)$, $d(-)$, and $D(+)$. Incidentally, the two different kinds of perithecia, one heterozygous for *downy* \times *non-downy*, and the other homozygous for *non-downy*, are in agreement with the rule that two nuclei associate at the initiation of the perithecium and divide conjugately until their products fuse in initiating the ascus.

Two asci from a cross of mycelium $1 \times F(-)$ were analysed. Both of these were from zygotes derived from $1(+)$ \times $F(-)$ nuclei.

All the thirteen asci tested from the $3 \times F(+)$ and from the $5 \times F(+)$ crosses proved to be from true crosses, and not from self-fertilisation of strains 3 and 5. This was to be expected in the $5 \times F(+)$ cross, for mycelium 5 had regularly reacted only as a $(-)$ strain.

All seven of the asci derived from the $1 \times \mathbf{F}(+)$ cross (tabulated above on p. 427) were tested for sex. Each ascus was tested by mating the mycelium derived from each of its four pairs of spores (1, 2, 3, 4) to three tester strains, namely, *fluffy* (+), *fluffy* (-), and the bisexual strain No. 1, making twelve tests for each ascus. The results of the tests are indicated by *P* for fertile and *N* for non-fertile combinations in the tabulation below:

	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
$\mathbf{F}(+)$	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>N</i>	<i>P</i>
$\mathbf{F}(-)$	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>N</i>	<i>P</i>
1 (\pm)	<i>P</i>	<i>P*</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P†</i>	<i>P</i>	<i>P†</i>	<i>P</i>	<i>N</i>	<i>P†</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P*</i>	<i>P</i>

The reactions to the $\mathbf{F}(+)$ and $\mathbf{F}(-)$ strains show that all the zygotes were heterozygous for the (+) and (-) genes, and that reduction was at the first division in four asci and at the second in three. In respect of their reactions to the very fertile $\mathbf{F}(+)$ and $\mathbf{F}(-)$ strains, these progeny of the $1 \times \mathbf{F}(+)$ mating do not show any irregularities. When mated to the heterokaryotic parent, perithecia were produced in all except two cases (marked by *N*). In the cases marked *P** only a few ripe ascospores were produced, and in the cases marked *P†* no ripe ascospores were produced, but perithecia containing unripe ascospores were found. These weak reactions may be considered to be expressions of the genes responsible for the sterility of the bisexual heterokaryon. The fact that parent 1 did not transmit to its progeny the unusual capacity for producing perithecia when mated either with $\mathbf{F}(+)$ or with $\mathbf{F}(-)$ mycelia corroborates the view that mycelium 1 is heterokaryotic.

Seven asci from the cross of $2 \times \mathbf{F}(+)$ were tested as to sex by crosses to the parental strains *fluffy* (+) and 2 (\pm) and in two cases to *fluffy* (-) also, with the results tabulated below:

	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
$\mathbf{F}(+)$	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>
$\mathbf{F}(-)$	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>
2 (\pm)	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>N</i>

The results of the tests with the very fertile *fluffy* mycelia were regular, giving both (+) and (-) reactions for the tested pairs of ascospores, with first-division reduction in six cases and second-division reduction in only one. However, mycelium 2 used as tester acted like simple (+) without indications of the (-) nuclei expected from its former behaviour. This lack of (-) nuclei is explained by the fact that the continued sub-culturing of the heterokaryon since the making of the original matings had given opportunity for somatic segregation to occur. Somatic segregation of a similar nature has been described by Dodge (1928) and Lindegren (1934).

However, the (+) component of strain 2 must have carried some sterility factors, since its reactions as (+) were below those of the normally fertile **F**(+) strain, and since the heterokaryon would otherwise have produced perithecia by itself. In order to demonstrate that sterility factors were actually responsible for the failure of bisexual heterokaryon 1 to produce perithecia, all possible crosses were made between the mycelia obtained from six asci, which resulted from crosses between this heterokaryon and another race. Four of these asci (1413, 1414, 1415 and 1416) were dissected from one perithecium produced by mating mycelium 1 with an **F**(+) mycelium. Since all the ascospores of these four asci produced *non-fluffy* mycelia, it was concluded this perithecium had been produced not by cross-mating but by self-fertilisation between the (+) and (-) components of mycelium 1 under the stimulus of the **F**(+) mycelium. The sexes of the sixteen mycelia coming from the four pairs of ascospores of each of the four asci were determined by mating with both **F**(+) and **F**(-) mycelia (part of tabulation on p. 430). No sterility was evident in these test matings for sex reactions because, although both parents had carried sterility factors, these were not capable of expressing themselves when the matings were made with the very fertile *fluffy* mycelia. The other two asci (1542 and 1543) had been dissected from a perithecium produced by mating 1 and **F**(-). The ascospores from these asci produced both *fluffy* and *non-fluffy* mycelia, and had therefore resulted from a zygote formed by a true cross between the (+) component of 1 and an **F**(-) nucleus. These should carry sterility genes from the 1(+) component of the sterile heterokaryon. Although matings were made in all combinations between the mycelia derived from these six asci, only those between eleven of the (+) and eleven of the (-) mycelia are shown in the following tabulation:

[illegible]

The above tabulation shows that only two (+) mycelia, 1414-5 and 1542-5, are fully fertile with all the (-) mycelia. It is significant that the most fertile (1414-5) came from an ascus which produced the highly sterile 1414-1 mycelium, and that one of the parents of fertile 1542-5 was the fertile *fluffy* mycelium. The wide range of genotypes with respect to sterility shows that more than one gene was responsible for this effect.

All the possible (+) by (+) and (-) by (-) matings were also made between the mycelia whose (+) by (-) tests are shown above. In spite of the reassortment of factors determining a positive physiological sex reaction (formation of perithecia) and the consequent variety of genotypes, none of these (+) by (+) and (-) by (-) crosses resulted in the production of perithecia. This seems evidence for the view that the (+) and (-) allelomorphs are the most important differentiators of the physiological sexes in *Neurospora*. In the genetical experiments it is possible to deal with this pair of differentiators as if they were two separate genes located at a distance of 6.5 cross-over units from the spindle fibre. This does not exclude the possibility that the locus 6.5 is simply the distance from the spindle fibre at which the paired chromosomes at meiosis change from two homologous strands which cross over freely with one another, or two non-homologous segments which do not cross over with each other.

DISCUSSION.

Genetical experiments on *Neurospora* are conducted with a technique which guarantees isolation for the selected strains, and prevents contamination or mingling. Continued inbreeding means that selection against factors producing self-sterility is extreme. Also, in the laboratory one may mate mycelia produced by ascospores from the same ascus. In nature, on the contrary, all the ascospores from a single perithecium probably germinate within a few centimetres of the perithecium from which they have been ejected. The numerous different mycelia immediately anastomose, and the nuclei representing the different genotypes derived from this perithecium are all commingled in the common cytoplasm of a coencytium which only later produces septations and partially isolates nuclei in groups of a few tens or hundreds. If any recombinational or mutant genotype emerging from the perithecium produces mycelial characteristics which would make it unable to compete successfully under natural conditions, this new genotype may be preserved indefinitely within the cytoplasm of the coencytium, where it is unable to develop the unfavourable somatic characteristic. This means that

practically every new gene can be preserved in the coencytium and recombined with other genes until by chance a genotype producing vigorous and favourable mycelial characters is finally obtained. The mutation rate in *Neurospora* might be relatively low without slowing evolution, because most of the mutants are preserved and may finally come to favourable expression in haploid mycelia which become separated from coencytia.

In nature the selection against self-sterility factors is not so severe as it is in the laboratory. When two mycelia of opposite sex, each of which produces an abundance of conidia when grown alone, are mated together in a test tube, they usually produce far fewer conidia in the joint culture. This scarcity of conidia is very marked if the mating results in a great abundance of perithecia. It appears that the mycelium can usually get only a limited amount of food from the substrate, and if this is used to produce perithecia fewer conidia are formed. The obvious biological advantage of the asexual conidia, which can be produced in a few hours, for the rapid dissemination of the species, as compared to ascospores, which may require weeks to produce, probably results in natural selection in favour of sterility factors. This means that any strain selected from nature, without the precaution of obtaining a single ascospore from an eight-spored ascus, will probably be a bisexual sterile heterokaryon. *Monilia sitophila* has been catalogued since 1843 as an imperfect fungus, and not until 1927 did Shear and Dodge find the perfect stage. Such cases are a good argument for the view that sterility factors abound in natural strains.

F. L. Stevens (1928) has shown that it is possible to produce perithecia in normally self-sterile ascomycete mycelia by exposure to ultra-violet light. These experiments can be explained on the theory that the strains with which he was working were bisexual heterokaryons, and the stimulus of the light disturbed the expression of the genes responsible for the self-sterility. If the strains were heterozygous, an entirely new and perhaps startling series of variations might have been promoted by the fertilisation-promoting action of the light. There may be a strong selection pressure for a particular kind of self-sterility gene, namely, one whose expression is most easily disturbed by changes in the external environment.

Moreau and Moruzi (1932 *a, b, c, d, e, f*) studied variation in a strain of *N. crassa* in which the ascospores were not cut out regularly, that is, one ascospore might include any number of the eight nuclei laid down in the ascus. Consequently, any number of ascospores from one to eight

might be formed in these asci. They discovered that matings between many mycelia obtained from these ascospores did not follow a bisexual scheme. This was to be expected in view of the original sterility factors shown to be present in their strain, by the hormone experiments, and the multinucleate nature of many of the ascospores. It also follows from the heterokaryosis that they should find "brusque" variation from heterothallism to homothallism in cultures from single multinucleate ascospores. Their experiments also show that the strains they used were heterokaryotic for conidial and aconidial nuclei. When these factors segregated, they produced ascospores from which either aconidial or conidial mycelia grew. Some of the conidial mycelia had the capacity to produce aconidial mycelia and *vice versa*. The phenotype of the parental heterokaryon would probably resemble that of the genotype which predominated in numbers. Somatic segregation could produce two or more kinds of conidia. All the variations reported by Moreau and Moruzi can be explained without assuming that point mutation has occurred.

The zygomycetes with practically no complex morphological structures are coenocytes. The abundance of species of this class show that a coenocytium has a survival value which makes it a competent competitor with organisms which produce elaborate structures for the performance of special functions. The sole elaborate structure which these coenocytia produce, namely reproductive organs, gives the clue to their survival value. A great many of the mutations in diploid organisms are probably lost, not because they are useless, but because there is not sufficient time available to test them out in all possible combinations. A coenocytic constitution compels a minimum of differentiation, but allows a maximum of genotypes to be produced and tested for survival value. The phylogenetic sequence in fungi seems to indicate that haploid plants with relatively elaborate structures and many cell walls have been evolved from haploid plants with relatively simple structures and very few cell walls.

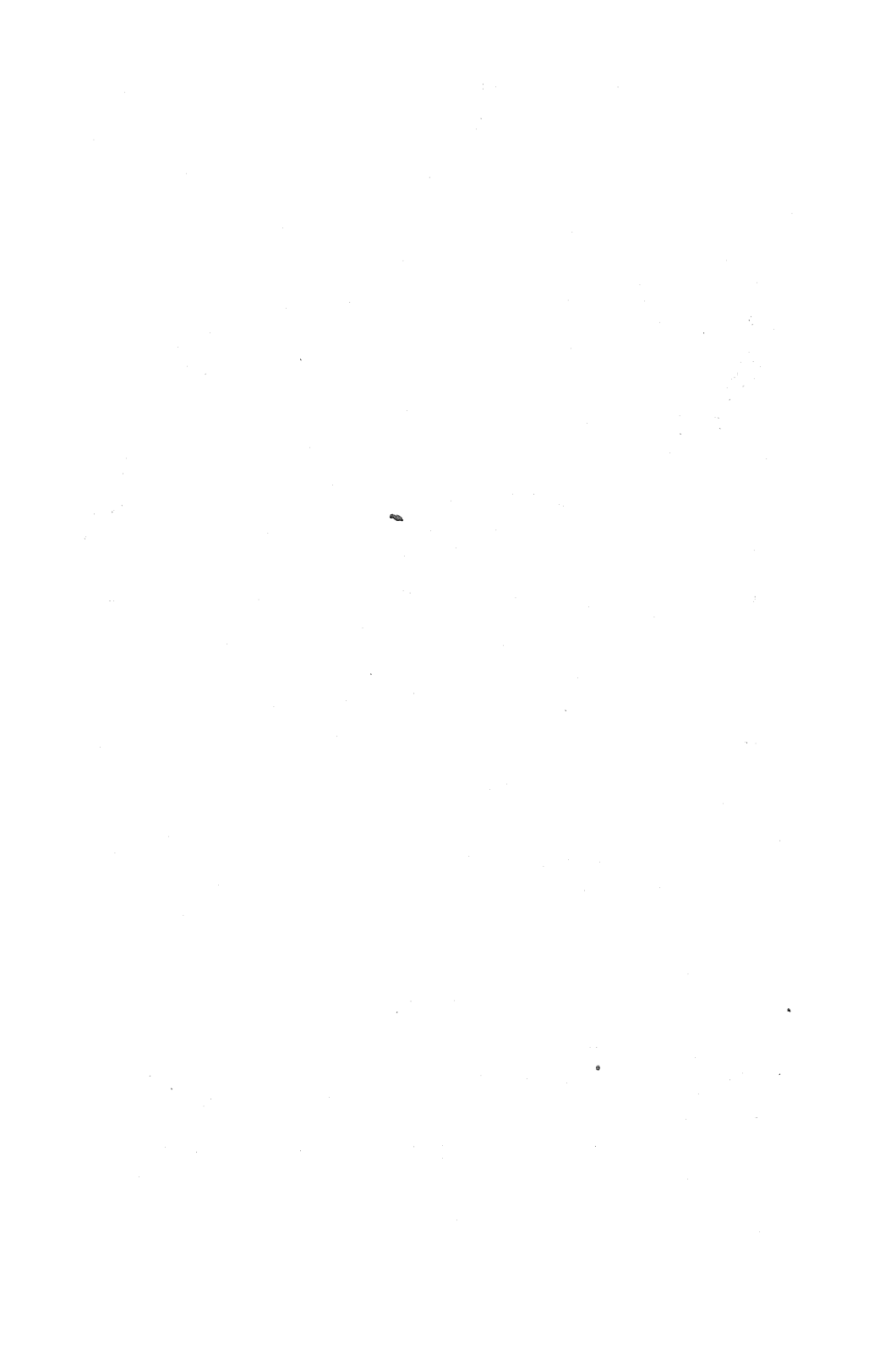
SUMMARY.

Certain *Neurospora* mycelia, incapable of producing perithecia when grown alone, were shown to contain both (+) and (−) nuclei, but these nuclei were incapable of reacting with each other to produce perithecia due to the presence of sterility factors. The possibility that such sterile bisexual heterokaryons are common among fungi is discussed.

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THE GENETICS OF COTTON.

PART X. THE INHERITANCE OF LEAF SHAPE IN ASIATIC *GOSSYPIUMS*.

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(With Twenty Text-figures.)

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INTRODUCTION.

THE shape of the leaf has been used by all workers on the taxonomy of the Asiatic species of *Gossypium* as a character of considerable, or even of major importance in classification. Since all the cultivated Asiatic *Gossypiums* are interfertile, and attempts are being made to obtain improved commercial varieties by selection among the progeny of interspecific crosses, a genetic study of the nature and behaviour of such a specific character as leaf shape seems to be of considerable importance.

PREVIOUS WORK.

The inheritance of leaf shape in Asiatic *Gossypiums* was first studied by Fyson (1908), who investigated crosses between *G. arboreum* and *G. herbaceum* var. *Wightiana*. Fyson classified his families by eye alone. He reports dominance of narrow leaf in all F_1 's, and gives the following results for three F_2 families:

Family	Narrow	Intermediate	Broad	Total
3	370	121	128	619
4	54	26	22	102
5	114	33	16	163
Total	538	180	166	884

Families 3 and 4 fit a simple Mendelian hypothesis fairly well, but there is a serious deficiency of recessives in family 5. In F_3 and later generations results consistent with simple Mendelian inheritance were obtained, if allowance is made for a certain amount of natural crossing.

Leake (1911) gives data on the inheritance of leaf shape in three crosses:

- (1) *G. arboreum* var. *Nanking*¹ \times *G. arboreum* (type 4 \times type 8).
- (2) *G. arboreum* \times *G. arboreum* var. *Nanking* (type 3 \times type 4).
- (3) *G. herbaceum* var. *Wightiana* \times *G. arboreum* (type 2 \times type 3).

He took three measurements on each of two typical leaves per plant:

(A) the length of the leaf from the point of insertion of the petiole to the tip of the middle lobe;

(B) the distance from the point of insertion of the petiole to the sinus between the middle lobe and the first lateral lobe; and

(E) the greatest width of the middle lobe.

He then took the factor $\frac{A-B}{E}$ as an index of the shape of the middle lobe.

G. arboreum (types 3 and 8) had a leaf factor greater than 3. *G. obtusifolium* var. *Wightiana* (type 2) and *G. arboreum* var. *Nanking* (type 4) had leaf factors less than 2. All F_1 's had leaf factors between 2 and 3.

In F_2 two of the crosses (type 4 \times type 8 and type 3 \times type 4) gave trimodal curves, with minima approximately at leaf factors 2 and 3. By dividing at these points three classes were obtained corresponding to the broad-leaved parent, the F_1 and the narrow-leaved parent. These

¹ For the sake of uniformity throughout the paper specific and varietal names have been altered to those of the present author's classification as included in Harland's (1932a) "The Genetics of *Gossypium*."

classes were obtained in approximately the proportions 1 : 2 : 1. A large F_3 was grown from the F_2 of type 3 \times type 4. Leake grouped together F_3 families having the same mean leaf factor. F_2 plants from the parental classes gave only progeny of their own class. F_2 plants with leaf factors between 2 and 3 segregated, giving a series of bimodal curves with a point of minimum frequency at or near leaf factor 2.0. There was no very definite evidence of a point of minimum frequency near leaf factor 3.0. In the group of families with the lowest family mean 43 per cent. of the plants had leaf factors below the point of minimum frequency, and in the group with the highest family mean only 9 per cent. of the plants had leaf factors below the point of minimum frequency. In the groups between the proportion of broad-leaved plants fell off as the family mean increased. As the individual F_3 families are not given separately, it is difficult to ascertain the reason for the variation in the proportion of broad-leaved segregates. It is clear, however, from the very high correlation between the means of the parents and the means of the offspring, in both homozygous groups and among the heterozygotes, that segregation occurred for minor factors affecting leaf shape as well as for the major factor, and it seems probable that the excess of broad-leaved segregates in groups of families with low means, and of narrow-leaved segregates in groups of families with high means, is due to the inclusion of homozygous broad-leaved families with a rather high mean leaf factor, and homozygous narrow-leaved families with a rather low mean leaf factor respectively. The two families with mean leaf factors of 2.05 and 2.06 respectively in Leake's Table XIV, are probably homozygous broad-leaved families of this type.

Leake's third cross (type 2 \times type 3) gave a monomodal curve which could not be analysed into components.

Kottur (1923) criticises Leake's "leaf factor" as being "more variable than the elements (length and breadth of middle lobe) of which it is composed," and because "the correlation between these elements is slight." The coefficients of variation of lobe length, lobe width, and leaf factor in a family of "*rosea*" given by Kottur are 13.2, 12.9 and 16.3 per cent. respectively. A difference of 3 per cent. in the coefficient of variation does not appear to be sufficient ground for discarding the leaf factor, and the correlation of $r = +0.28$ between leaf length and lobe width given by Kottur for "*rosea*" is certainly not negligible. From the data given in Kottur's Fig. 5, the correlation between lobe length and lobe width in Dharwar No. 1, and in F_1 of *Dharwar* \times *rosea* have been determined, and gave $r = +0.56$ and $r = +0.47$ respectively.

That the leaf factor increases considerably with increasing vigour of the plant is beyond dispute, but in view of the considerable positive correlations between lobe length and lobe width in both parents and in the F_1 of Kottur's cross, it must be concluded that a leaf factor will provide a better estimate of leaf shape than will the elements of which it is composed. Kottur gives no correlation tables of length and width of lobe for his F_2 and later generations, and his lobe-length and lobe-width frequency arrays provide no justification whatever for the analysis to which he subjects them. They will not, therefore, be further considered here.

METHODS.

In view of the success of Leake's "leaf-factor" methods in resolving his segregating F_2 's into separate leaf-shape classes, and in view of the weakness of Kottur's criticism (see above), it was decided at the beginning of this investigation to employ some similar index as an estimate of leaf shape. The three measurements chosen by Leake—leaf length (A), the distance from the insertion of the petiole to the sinus between the middle lobe and the first lateral lobe (B), and the greatest width of the middle lobe (E), were used, and means were calculated from measurements on five leaves on the main stem of each plant. Sinus length (B) and lobe width (E) were found to be highly correlated. On 15 leaves from a single plant of *G. arboreum* the correlation was $r = +0.66$ ($P = 0.01$). In a family of 130 plants of *G. arboreum* (G.S.) the correlation was $r = +0.51$ (P very small), and in an F_2 of *G. arboreum* \times *G. arboreum* var. *Nanking* segregating for leaf shape it was $r = +0.91$ (P very small indeed). It was therefore decided not to subtract the sinus length (B) from the leaf length (A), but to calculate two indices:

Index A , $\frac{\text{the length of the leaf } A}{\text{the sinus length } B}$, and Index B , $\frac{\text{the length of the leaf } A}{\text{the lobe width } E}$.

These are, of course, highly correlated, since B and E are correlated and A is common to both. Then on a correlation table of the two indices, the plants of segregating families were found to be grouped into definite classes. Correlation tables of indices A and B have been compiled and examined for all families here reported. One is reproduced here (Table II), but in order to save space, the data for most families are given in the form of frequency arrays of the mean of indices A and B (hereafter referred to as the "Mean Index"). Leake (1911) considered index A , but decided that it was too variable to be useful. It certainly varies more than index B , and gives more overlapping between classes, but has

proved very useful as a check on index B , in the first place in the detection of errors of measurement or computation, and in the second place as an additional estimate of leaf shape to decide the constitution of doubtful plants near the point of minimum frequency.

It may be mentioned here, that for purposes of comparison with Leake's work, the mean index used in this paper may be taken to be equal to Leake's leaf factor + 1, and to be correlated with Leake's leaf factor to the extent of about $r = +0.9$.

It will be shown that the shape of the leaf is controlled by a series of five multiple allelomorphs, for which the following symbols will be used:

Laciniated	L^L
Arboreum	L
Recessive Broad	l
Mutant Broad	L^B
Mutant Intermediate	L^I

I. INTERSPECIFIC AND INTERVARIETAL CROSSES.

MATERIAL.

The cultivated Asiatic *Gossypiums* are divided by Watt (1907) into three groups on their leaf shape:

(1) "Leaves two-thirds palmately (sometimes almost pedately) 3-7 lobed, lobes curvilinear, bristle tipped, and base usually distinctly cordate...." *G. arboreum* and its varieties.

(2) "Leaves half cut into 3-5 (mostly 3) lobes...lobes deltoid oblong acute or acuminate, base usually only slightly cordate...."

G. Nanking and its varieties.

(3) "Leaves less than half cut into 5 (more rarely 3 or 7) lobes, which are constricted below (ogee shaped) obtuse or acute, distinctly cordate...." *G. obtusifolium* and *G. herbaceum* and their varieties.

In the classification included in "The genetics of *Gossypium*" (Harland, 1932*a*) these four species are reduced to two, *G. Nanking* being reduced to a variety of *G. arboreum*, and *G. obtusifolium* to a variety of *G. herbaceum*.

Evidence in support of this classification was presented in previous papers (Hutchinson 1931, 1932*a*, 1932*b*), and further evidence is offered below.

A series of crosses was made between representative types of these two species and their more important varieties.

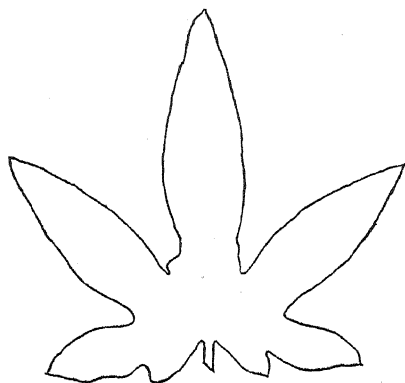


Fig. 1.

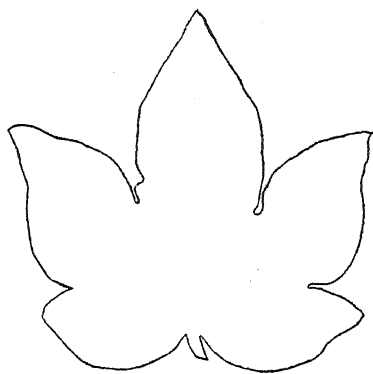


Fig. 2.

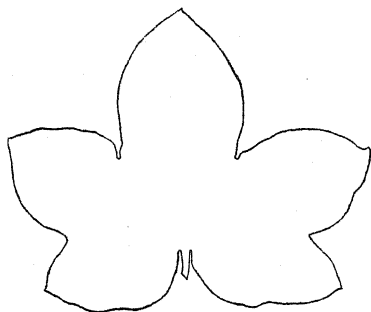


Fig. 3

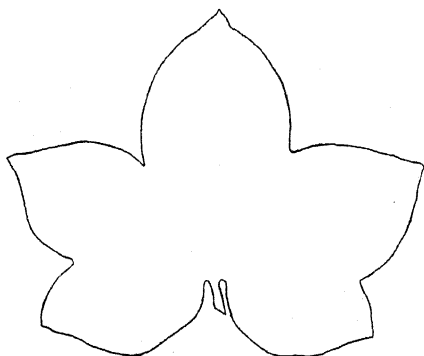


Fig. 4.

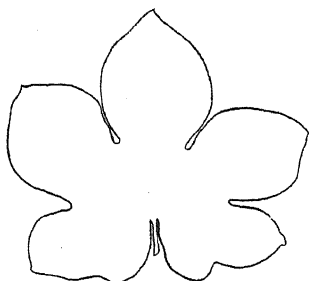


Fig. 5.

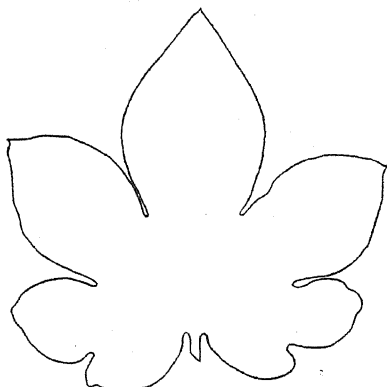


Fig 6.

Fig. 1. Leaf outline of Cawnpore White.

Fig. 2. Leaf outline of Million Dollar.

Fig. 3. Leaf outline of *G. arboreum* var. *soudanensis* (Abu Hareira).

Fig. 4. Leaf outline of *G. herbaceum* (N289).

Fig. 5. Leaf outline of *G. herbaceum* var. *africana* (Moamba).

Fig. 6. Leaf outline of *G. herbaceum* var. *Wightiana* (1027 ALF).

✓ The following types were used as parents:

(1) *G. arboreum*. "Leaves two-thirds palmately lobed." The Linnean type of *G. arboreum* was represented in these experiments by a strain known as 24-2. The sympodial form *neglecta* was represented by three strains, Cawnpore Yellow, A13, and Chickenfoot. The two sympodial forms *rosea* and *sanguinea* were represented by strains known as Cawnpore White and G.S. respectively.

The leaves of these six strains were very similar in shape. The outline of a leaf of Cawnpore White is given in Fig. 1, and may be taken as typical of the six *arboreum* strains used.

(2) *G. arboreum* var. *Nanking*. "Leaves half cut into 3-5 lobes." Three strains of *G. arboreum* var. *Nanking* were used, 1304, Burma Ghost and Million Dollar. The outline of a leaf of Million Dollar is given in Fig. 2. Leaves of Burma Ghost and 1304 were similar, but a little more deeply cut.

A strain of *G. arboreum* var. *soudanensis* known as Abu Hareira was also used. Leaves of this plant had somewhat rounded lobes, intermediate between *G. arboreum* var. *Nanking* and *G. herbaceum*. An outline of the leaf is given in Fig. 3.

(3) *G. herbaceum*. "Leaves less than half cut." The four strains of *G. herbaceum* used had leaves almost exactly alike. All had large, very broad, flat leaves, with rounded lobes. See outline in Fig. 4.

(4) *G. herbaceum* var. *Wightiana* and *G. herbaceum* var. *africana*. "Leaves less than half cut." These are the varieties classified as *G. obtusifolium* by Watt.

Two strains of *G. herbaceum* var. *Wightiana* were used, Wagad and 1027. The leaves were large and rumpled with lobes rather more pointed than in *G. herbaceum* (type) and much constricted at the base. See outline in Fig. 6.

A strain of *G. herbaceum* var. *africana* was used, which was known as O7, and was obtained from Moamba, Portuguese East Africa. It had small, flat smooth leaves with rounded lobes, much constricted at the base. See outline in Fig. 5.

Frequency arrays are given in Table I of the mean indices of the types employed as parents.

EXPERIMENTS.

G. arboreum × *G. arboreum* var. *Nanking*.

(1) Million Dollar × Cawnpore.

Million Dollar is a Chinese variety. Two types selected from a mixed lot of Cawnpore cotton were used, (1) Cawnpore Yellow (*neglecta*) and (2) Cawnpore White (*rosea*). Cawnpore Yellow had on the whole somewhat narrower-lobed leaves than Cawnpore White, but both contrast strongly with the broad-lobed Million Dollar (cf. Figs. 1 and 2). Both F_1 's were strictly intermediate between the parents in leaf shape, the mean leaf index of the F_1 's being near the arithmetic mean of the mean indices of the parents. F_2 's were grown and a back-cross of (Million Dollar × Cawnpore White) × Cawnpore White, and 31 F_3 families from the F_2 of Million Dollar × Cawnpore Yellow. Mean index distributions for the F_2 's and back-cross are given in Table III. F_2 's were grown in 1926-7 and 1927-8 from the same F_1 of Million Dollar × Cawnpore Yellow, and are given separately to show how little variation there is from season to season. A correlation table is given (Table II) for leaf indices *A* and *B* for the 1927-8 F_2 , to show the close association between the two indices. The F_2 falls into two classes on the correlation table, with an area of low frequency about 3.2×3.2 . The mean index distributions are consequently bimodal with a minimum point at 3.2. Dividing at this point and omitting plants with a mean index of 3.2 gives:

	Narrow and intermediate	Broad	Total
Observed	331	111	442
Expected	331.5	110.5	442

The minimum point in the distribution of Million Dollar × Cawnpore White falls at 3.1 (Table III). Omitting plants with mean index 3.1 and dividing gives:

	Narrow and intermediate	Broad	Total
Observed	973	316	1289
Expected	966.75	322.25	1289

The back-cross to Cawnpore White gave narrows and intermediates only.

In the F_3 of Million Dollar × Cawnpore Yellow three types of behaviour occurred. (1) Seven F_2 plants gave broad-leaved progeny only. Except among the progeny of F_2 plant 469 all plants in these families had mean indices below 3.5. It is probable that a certain amount of natural crossing occurred on plant 469. (2) Eight F_2 plants gave narrow

and intermediate-leaved progeny only. (3) Sixteen F_2 plants gave progeny which segregated into narrow plus intermediate, and broad. Aggregate frequency arrays for the three types of behaviour are given in Table III. The point of division of the frequency array of the sum of the segregating families is at 3.0. Dividing the individual families at that point, and omitting all frequencies at 3.0, gave:

F_2 plant	{ number	460	200	383	466	454	199	385	400
	{ mean index	2.4	3.2	3.6	3.6	3.6	3.6	3.7	3.8
F_3 progeny	{ narrow	14	9	11	30	65	37	61	121
	{ broad	8	6	3	8	9	15	12	41
	{ Total	22	15	14	38	74	52	73	162
F_2 plant	{ number	451	396	376	405	390	459	389	378
	{ mean index	3.9	4.0	4.0	4.0	4.0	4.0	4.2	4.4
F_3 progeny	{ narrow	80	35	85	163	141	22	34	16
	{ broad	31	16	31	49	29	4	5	8
	{ Total	111	51	116	212	170	26	39	24

Giving in all:

	Narrow	Broad	Total
Observed	924	275	1199
Expected	899.25	299.75	1199

$$\chi^2 = 2.82, \quad n = 1, \quad P = 0.1.$$

The fit to the expected 3 : 1 ratio is rather poor. The minimum point varied somewhat from family to family, as would be expected with a quantitative character, especially if segregation of modifying factors is taking place. The point of minimum frequency was more often above 3.0 than below, and if the division had been taken at the minimum point for each family, the fit to a 3 : 1 ratio would have been improved.

The mean index distributions of the three classes of F_2 plants used as parents of F_3 families are given in Table III. Homozygous narrow-leaved plants are designated **LL**, heterozygotes **Ll**, and homozygous broad-leaved plants **ll**. There is very little overlapping between the distributions of **Ll** and **ll**. The heterozygote at 2.4 and the homozygous broad at 4.1 appear to have been errors in recording.

The distributions of **LL** and **Ll** overlap more, and in the progeny of **LL** plants, plants with mean indices as low as 3.0 occur, so that it is clear that a trimodal curve divisible in the proportions 1 : 2 : 1 could not be expected. The totals 8**LL** : 16**Ll** : 7**ll**, agree well with the expected proportions 1 : 2 : 1.

(2) Burma Ghost \times Cawnpore White.

The same strain of Cawnpore White was used to cross with a slightly narrower leaved *G. arboreum* var. *Nanking* known as Burma Ghost. The

F_1 was again strictly intermediate (Table III). An F_2 was grown and a series of F_3 families. The F_2 gave a bimodal curve for mean index very similar to those obtained from the Million Dollar \times Cawnpore crosses (Table III), with the point of minimum frequency at 3.0. Omitting this frequency and dividing gives:

	Narrow and intermediate	Broad	Total
Observed	288	83	371
Expected	278.25	92.75	371

There is a slight deficiency of broad-leaved segregates, but from the distributions of the parents of F_3 families given at the bottom of Table III, it will be seen that most of the plants tested with a mean index of 3.0 and some of those with a mean index of 3.1 proved to be homozygous **ll**. In F_3 the expected types of behaviour appeared in the proportions 18**ll** : 45**Ll** : 11**LL**.

Aggregate frequency arrays for the three types are given in Table III.

The distribution of the **LL** parents overlapped that of the **Ll** parents considerably (Table III). The overlapping between heterozygotes and homozygous **ll** was again slight. The frequency distribution for mean index on the total of the segregating families gave a bimodal curve with a minimum at 3.1. Dividing and omitting frequencies at 3.1 gave:

	Narrow and intermediate	Broad	Total
Observed	633	207	840
Expected	630	210	840

a good fit to the expected 3 : 1 ratio.

Examination of the frequency arrays of individual families, however, revealed a considerable amount of variation in the position of the point of minimum frequency. It ranged as low as 2.9 and as high as 3.5. This is consistent with the results from homozygous **ll** families, where the upper limit of the frequency arrays ranged from 2.9 to 3.6.

Below are given the results obtained by dividing each segregating F_3 family at the appropriate minimum point.

Family	131	114	96	68	58	127	110	93	37	26	54
Parental index	3.0	3.1	3.1	3.1	3.1	3.1	3.2	3.2	3.2	3.2	3.2
Minimum frequency	3.2	2.9	3.2	3.1	3.3	3.0	2.9	2.9	3.0	3.0	3.5
Narrow	9	12	28	24	19	9	7	9	21	13	34
Broad	4	4	12	7	10	4	5	2	3	3	16
Total	13	16	40	31	29	13	12	11	24	16	50
Family	104	55	83	71	75	60	62	27	19	24	148
Parental index	3.3	3.3	3.3	3.3	3.3	3.4	3.4	3.4	3.4	3.4	3.4
Minimum frequency	3.2	3.4	3.2	3.5	3.3	3.0	2.9	3.1	2.9	3.0	3.1
Narrow	8	12	19	6	9	10	12	7	15	12	9
Broad	5	11	7	4	1	2	7	3	2	1	3
Total	13	23	26	10	10	12	19	10	17	13	12

Family	139	123	126	113	95	100	34	145	80	10	158
Parental index	3.4	3.4	3.4	3.4	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Minimum frequency	3.3	3.1	3.3	3.2	3.4	3.1	3.3	3.2	3.1	3.2	3.3
Narrow	6	14	8	17	32	34	15	18	10	7	13
Broad	4	4	3	5	8	7	5	3	1	4	3
Total	10	18	11	22	40	41	20	21	11	11	16
Family	120	76	117	36	21	111	108	152	17	128	63
Parental index	3.6	3.6	3.6	3.7	3.7	3.7	3.8	3.8	3.8	3.8	3.9
Minimum frequency	3.2	3.3	3.5	3.3	3.3	3.1	3.1	3.0	2.9	3.3	3.2
Narrow	7	12	18	12	11	16	24	21	7	21	17
Broad	4	8	5	5	4	6	8	4	4	6	7
Total	11	20	23	17	15	22	32	25	11	27	24

Sum totals: Observed 644 narrow: 224 broad
Expected 651 narrow: 217 broad.

Testing for homogeneity, $\chi^2=36.8$, $n=43$, and $P=0.5$.

The families may therefore be taken as homogeneous in regard to the main factor.

(3) *Sanguinea* \times Abu Hareira.

G.S. 2, the *sanguinea* used, is very similar to Cawnpore in leaf shape (see Fig. 1). Abu Hareira (*G. arboreum* var. *soudanensis*) has somewhat broader leaves (see Fig. 3) with rather more rounded lobes than the type of *G. arboreum* var. *Nanking* (e.g. Million Dollar or Burma Ghost) (Fig. 2). The F_1 was intermediate. An F_2 and back-crosses to both parents were grown. Mean index distributions are given in Table III. In F_2 and the back-cross to Abu Hareira bimodal curves were obtained. In F_2 the minimum occurred at mean index 3.0, and in the back-cross at mean index 2.8. Dividing at these points gave:

		Narrow and intermediate	Broad	Total
F_2	Observed	190	74	264
	Expected	198	66	264
Back-cross	Observed	128	141	269
	Expected	134.5	134.5	269

In the back-cross to *sanguinea*, another plant, G.S. 1, of the same *sanguinea* strain was used, and half the progeny were abnormal "crumpled" (see Hutchinson, 1932a). All the normal segregates had mean leaf indices above 3.0.

These results confirm Leake's conclusion that there is a single factor difference between the narrow *arboreum* type of leaf and the broad *Nanking* type.

G. arboreum \times *G. herbaceum*.

(1) 24-2 \times N454.

24-2 is a typical *arboreum*, with a leaf closely similar to that shown in Fig. 1. N454 is a typical *herbaceum*, with a leaf like that shown in Fig. 4,

very broad and with rounded lobes, and consequently a very low mean index (about 1.9, see Table I). F_1 was intermediate (Table IV). An F_2 was grown and a back-cross to 24-2. Mean index distributions are given in Table IV. The F_2 gave a bimodal curve with a minimum at 2.7. Dividing at this point gave:

	Narrow and intermediate	Broad	Total
Observed	288	84	372
Expected	279	93	372

There is a second minimum at 3.9, and the mean index distribution for the back-cross to 24-2 is bimodal, with a minimum at 4.1, and no plants with a mean index less than 3.1. In the F_2 there were only 54 plants out of 381 with a mean index above 3.9, and in the back-cross only 23 plants out of 67 with a mean index above 4.1. No doubt practically all plants in these classes were homozygous narrow, but they do not fit the expectation for simple monofactorial segregation, and not much significance can be attached to the minima at these points.

(2) Cawnpore White \times N289.

The leaf of Cawnpore White is shown in Fig. 1, and that of N289 in Fig. 4. Mean indices for the parents are given in Table I. F_1 was again intermediate (Table IV). An F_2 and back-crosses to both parents were grown. Mean index distributions are given in Table IV. The F_2 and the back-cross to N289 gave bimodal distributions. The minimum point for the F_2 is at 2.9. The back-cross to Cawnpore White gave only one plant with mean index below 2.8. There is no evidence of a separation into classes in the back-cross to Cawnpore White. The minimum point in the mean index distribution for the back-cross to N289 might be either at 2.8 or 3.0. Since the back-cross to Cawnpore White gave a distribution descending as low as 2.8, it is more reasonable to divide at that point.

Dividing the F_2 and back-cross to N289 at the minimum points gives:

		Narrow and intermediate	Broad	Total
F_2	Observed	90	27	117
	Expected	87.75	29.25	117
Back-cross	Observed	99	90	189
	Expected	94.5	94.5	189

An F_2 was also grown from seed that had been in store for some time, and which germinated very badly. Of the 123 plants obtained, 105 had narrow leaves and 18 only had broad leaves. In another F_2 there was a very heavy (about 40 per cent.) seedling mortality, owing to unfavourable conditions soon after planting. Of the 71 survivors in this family, 59 had

narrow leaves and only 12 had broad leaves. Apparently the viability of seeds and the vigour of seedlings is greater among narrow-leaved than among broad-leaved genotypes.

A small F_3 was grown from open pollinated seed. A certain amount of natural crossing had taken place, but five families, the progeny of broad-leaved F_2 plants, bred true to broad, nine families from intermediate F_2 plants behaved as heterozygotes, and gave approximately 3 narrow and intermediate : 1 broad, and four families from narrow-leaved F_2 plants, bred true to narrow leaf. Aggregate frequency arrays for the three types are given in Table IV.

(3) $H9 \times 24-2$, $H9 \times$ Cawnpore White, and $H10 \times$ Cawnpore White.

$H9$ and $H10$ are typical herbaceums with leaves similar to that shown in Fig. 4. The three F_1 's, $H9 \times 24-2$, $H9 \times$ Cawnpore White, and $H10 \times$ Cawnpore White were intermediate between the parents, having mean indices near the mean of the parental indices. Each F_1 was back-crossed both to $H10$ and to Burma Ghost. Mean index distributions are given in Table IV. All are bimodal, and do not go above 3.9. The back-crosses to Burma Ghost (*G. arboreum* var. *Nanking*) may be divided between 2.8 and 2.9 and give:

	Intermediate	Broad	Total
$(H9 \times 24-2) \times B.G.$	8	8	16
$(H9 \times C.W.) \times B.G.$	30	26	56
$(H10 \times C.W.) \times B.G.$	30	28	58
Total	68	62	130
Expected	65	65	130

The back-crosses to $H10$ (*G. herbaceum*) may be divided at 2.5 and give:

	Intermediate	Broad	Total
$(H9 \times 24-2) \times H10$	53	43	96
$(H9 \times C.W.) \times H10$	10	6	16
$(H10 \times C.W.) \times H10$	32	40	72
Total	95	89	184
Expected	92	92	184

These results show that the same factor is responsible for the difference in shape between the *arboreum* leaf and the *herbaceum* leaf as between the *arboreum* leaf and the *Nanking* leaf.

The $H9$ and $H10 \times$ *arboreum* back-crosses show clearly the effect of those modifying factors which cause the difference in shape between *Nanking* and *herbaceum* leaves. A back-cross of an *arboreum* \times *herbaceum* heterozygote to *Nanking* gives a mean index distribution about 0.25 higher in the scale than the corresponding back-cross to *herbaceum*.

(4) *G. herbaceum* var. *Wightiana* \times *G. arboreum*.

Two forms of *G. herbaceum* var. *Wightiana*—Wagad and 1027—were crossed with Cawnpore White. A leaf of 1027 is shown in Fig. 5, and the leaf of Wagad is similar. Mean indices for the parents are given in Table I. F_1 's were intermediate (Table IV). An F_2 was grown from Wagad \times Cawnpore White, and an F_2 and back-crosses to both parents from 1027 \times Cawnpore White. Mean index distributions are given in Table IV. The distributions of the F_2 's and the back-cross (Cawnpore White \times 1027) \times 1027 are bimodal, with minima at 2.9 or 3.0. Dividing at these points gives:

			Narrow and intermediate	Broad	Total
C.W. \times Wagad	F_2	Observed	43	16	59
		Expected	44.25	14.75	59.0
C.W. \times 1027	F_2	Observed	101	36	137
		Expected	102.75	34.25	137
(C.W. \times 1027) \times 1027		Observed	32	40	72
		Expected	36	36	72

The back-cross (Cawnpore White \times 1027) \times 1027 gave nothing but intermediates and narrows, with no plant with a lower mean index than 3.0.

Besides the main difference in laciniation between *G. arboreum* and *G. herbaceum* var. *Wightiana*, there were minor differences which were not amenable to measurement. In particular, *Wightiana* leaves are usually rumpled, whereas *arboreum* leaves are flat. In both F_1 's the leaves were nearly flat. In the F_2 's the rumpling character appeared in varying degrees on a number of plants, and was quite independent of the main factor. In the back-cross of (Cawnpore White \times 1027) \times Cawnpore White all plants had flat or nearly flat leaves. In the back-cross (Cawnpore White \times 1027) \times 1027 all plants had more or less rumpled leaves.

(5) *G. arboreum* \times *G. herbaceum* var. *africana*.

A form of the wild *G. herbaceum* var. *africana* from Moamba, Portuguese East Africa, known as O7, was crossed by "Chickenfoot," a Chinese *arboreum*. *Africana* has a small leaf, with very broad lobes, very much rounded and deeply constricted at the base (see Fig. 6). Two F_1 plants had mean indices of 3.1, intermediate between the parents, with the lobes of the leaf only moderately constricted. An F_2 was grown, and gave 42 narrow and intermediate-leaved plants : 14 broad leaved, or 3 : 1. A mean index distribution for the F_2 is given in Table IV. Both narrow and broad-leaved classes extend considerably beyond the ranges of the

corresponding parental classes. In both broad and narrow classes, index *A* was in nearly all cases higher than index *B*, whereas in *arboreum* \times var. *Nanking* crosses the two indices are about equal. The difference is due to the effect of genes causing constriction of the base of the lobe, which at the same time reduce the sinus length.

In this cross again the segregation of the main gene **L** is clear, and the influence of minor gene differences such as those affecting leaf size and the constriction of the base of the lobe is shown in a minor shift in the distribution of one of the indices, and the extension of the frequency array beyond the limits of the parents.

Crosses between broad-leaved types.

A series of crosses between broad-leaved types was investigated in order to obtain further information on the differences between different broad-leaved species. Mean index distributions are given in Table V.

(1) *G. arboreum* var. *Nanking* \times *G. herbaceum*.

Two crosses were investigated: (1) Burma Ghost \times N289, F_2 and back-crosses to N289 and 1304, and (2) H9 \times 1304 back-crosses to 1304 and H10. 1304 is a *Nanking* extracted from the F_3 of Cawnpore White \times Burma Ghost. F_1 's were intermediate and F_2 's and back-crosses gave smooth mean index distributions. Burma Ghost \times N289 F_2 and back-crosses gave some plants with mean indices higher than the higher parent (see Table V). These plants were examined in the field, and it was concluded that the whole of the variation was due to segregation and recombination of modifying factors on the basic **II** genotype. The difference between back-crosses to *Nanking* and to *herbaceum* in both Burma Ghost \times N289 and H9 \times 1304 emphasises the effect of modifiers. In both cases the back-cross to *herbaceum* gave on the whole considerable broader leaved plants than the back-cross to *Nanking* (see Table V), as was also the case in back-crosses of *arboreum* \times *herbaceum* to *herbaceum* and to *Nanking* (see Table IV).

(2) *G. arboreum* var. *Nanking* \times *G. herbaceum* var. *Wightiana*. Million Dollar \times 1027.

There was very little measurable difference between the leaves of the two parent types, and the F_1 , F_2 and back-cross distributions (see Table V) were similar to those of the parents, except that the range was somewhat greater in F_2 and back-crosses, no doubt as the result of segregation of modifying factors.

Mean leaf index distributions of F₁'s, F₂'s and back-crosses between broad leaved types.

[illegible]

Mean leaf index distributions of F_1 's, F_2 's and back-crosses of Burma Lacinated \times Mutants.

[illegible]

The parents differ considerably in the form of the base of the leaf, a seven-lobed leaf being fairly common, though by no means universal, on the main stems of 1027, and unknown on Million Dollar. The lateral and basal lobes are more rounded on 1027 than on Million Dollar, and the leaves of 1027 are rumpled. The F_1 had a flat leaf rather like Million Dollar, but occasionally seven lobed. In F_2 most of the plants had flat or nearly flat leaves, usually like Million Dollar, and only on a very small proportion of the F_2 plants were the leaves more like 1027 than the F_1 . All plants in the back-cross to Million Dollar had leaves like Million Dollar. In the back-cross to 1027 a range of types was found grading from true 1027 leaves to the F_1 type, but true Million Dollar type leaves did not occur.

(3) *G. herbaceum* type \times var. *africana*. N 289 \times O7.

Two F_1 's had mean indices of 2.2 and 2.4. A mean index frequency array for the F_2 is given in Table V. Only broad-leaved types occurred, varying in such minor characters as the degree of constriction of the base of the lobe, and the size of the leaf.

II. THE MULTIPLE ALLELOMORPH SERIES AND MUTABLE GENES.

MATERIAL.

In the course of the experiments on leaf shape, two strains were discovered which gave unexpected results. These were:

(1) A strain of *G. arboreum* known as Burma Laciniated with extremely laciniated leaves (see Fig. 7). The strain originated as a selection from a bulk lot obtained by Dr S. C. Harland, from Mahlaing, Burma, in 1925.

In 1929 a branch appeared on a Burma Laciniated plant, which bore broad leaves similar to those of *G. arboreum* var. *Nanking*, but somewhat smaller (see Fig. 8). A graft was taken and selfed seed obtained. The broad-leaved mutant bred true immediately. In 1930 a number of broad-leaved mutants were observed on plants of Burma Laciniated and its hybrids. These were of two types, some being like the original mutant, and others having rather narrower leaves (see Fig. 9). A strain was established from one of these latter which appeared on a graft from the Burma Laciniated plant which gave rise to the original mutation. This also bred true from the start. The two mutants were called Mutant Broad and Mutant Intermediate respectively, and were given the serial numbers A9 and A20.

- Burma Lacinated and its mutants bred true for brown lint.
 Two other mutants were used extensively in the experiments:
 (a) An F_1 plant of Burma Lacinated \times 1304 gave rise to a single



Fig. 7.

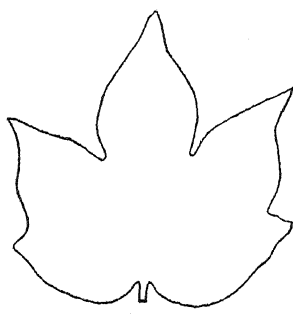


Fig. 8.

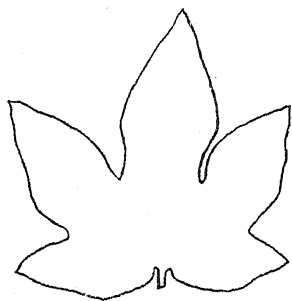


Fig. 9.

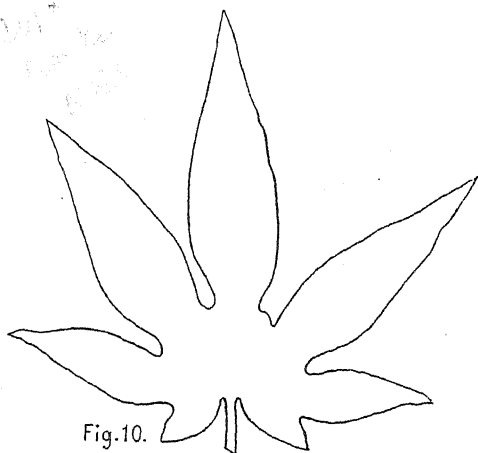


Fig. 10.

Fig. 7. Leaf outline of Burma Lacinated.

Fig. 8. Leaf outline of Mutant Broad.

Fig. 9. Leaf outline of Mutant Intermediate.

Fig. 10. Leaf outline of *cernuum*.

broad-leaved branch with a mean index of 2.5 compared with 7.0 on the rest of the plant. This was known as "985 Mutant."

(b) In a back-cross of (Cawnpore White \times N289) $8 \times$ Burma

Laciniated in which all plants should have had laciniated leaves, a single plant with broad leaves appeared. As different branches appeared to differ somewhat in leaf shape, 11 grafts were taken. The plant was then cut back. The cotyledonary nodes gave rise to two branches which bore laciniated leaves. Grafts were also obtained from these, and gave mean indices of 4.8 and 5.2. Mutation had apparently occurred in the main stem just above the cotyledonary nodes. The mean indices of broad-leaved grafts ranged from 2.3 to 4.3 (see Table XIII). This mutant was known as "814 Mutant."

(2) *G. arboreum* var. *assamica*. The strain used was obtained from India, by Dr H. M. Leake, and was grown under the name of *G. cernuum*. Typical *cernuum* has extremely laciniated leaves, very similar to those of Burma Laciniated. The strain was said to be mixed when first grown in Trinidad, although it bred true for the typical *cernuum* boll. As the boll size was the most interesting feature of the variety, no particular attention was paid to the leaf shape until later, when it was found that some *cernuum* hybrids gave extremely laciniated segregates in F_2 , and others gave no plants with leaves more laciniated than typical *arboreum*. The reselected *cernuum* then growing in the variety rows was found to have leaves similar to typical *G. arboreum*, but rather longer and with broader lobes (see Fig. 10). *Cernuum* has white lint.

All *cernuum* crosses here reported were made with the reselected *cernuum*, except the original cross of *Cernuum* \times Cawnpore White, and *Cernuum* \times 5F.

Frequency arrays of mean index for the types used are given in Table I.

EXPERIMENTS.

(1) *The constitution of Burma Laciniated.*

The original Burma Laciniated was crossed with an *arboreum* (Cawnpore White) and a *Nanking* (1304), with white lints, and a *herbaceum* (N289), with brown lint. In addition, a cross was made between a plant of hybrid origin which had inherited laciniated leaf from Burma Laciniated, and Chickenfoot. The F_1 's were similar to Burma Laciniated in leaf shape, but rather broader, and had brown lint. Back-crosses to Cawnpore White and to 1304 were grown, and F_2 's of the Burma Laciniated by recessive broad crosses. Frequency arrays of mean index are given in Table VI. In the crosses between Burma Laciniated and Cawn-

pore White and 1304, the arrays of brown and white-linted segregates are given separately.

Comparison of the F_1 arrays with the array of Burma Laciniated given in Table I shows that dominance is not complete. Single-factor segregation occurred for leaf shape in all families. Single-factor segregation also occurred for lint colour. (Burma Laciniated \times Cawnpore White) \times Cawnpore White may be divided at mean leaf index 4.3 corresponding to the upper limit of the distribution of Cawnpore White given in Table I. The frequencies of the four classes are:

Brown laciniated	White laciniated	Brown <i>arboresum</i>	White <i>arboresum</i>	Total
42	14	16	40	112

There were equal numbers of laciniated and *arboresum* types, and 58 brown : 54 white. The brown lint gene, K , is evidently linked with the leaf shape gene L^L . Cross-over classes amounted to 30 plants out of 112, giving 26.8 per cent. crossing-over. The *arboresum* class had a leaf-shape distribution running rather low compared with the Cawnpore White parent, but the plants were grown in very dry weather, and the leaves were exceptionally short.

The F_1 plant of Burma Laciniated \times 1304 which was used as parent of the back-cross families was plant 985, which gave a mutant branch, the behaviour of which will be considered later.

The F_2 and the back-cross to 1304 gave broad and laciniated and brown-linted and white-linted segregates. The distinction between broad and laciniated was very clear, broad-leaved plants having leaf indices up to 3.1 and laciniated from 4.4 upwards.

	Brown laciniated	White laciniated	Brown broad	White broad	Total
F_2	15	1	1	5	22
$F_1 \times 1304$	39	22	16	32	109

Brown and white and laciniated and broad occurred in the expected 3 : 1 proportions in F_2 , and 1 : 1 in the back-cross. Brown and laciniated were again linked. In the back-cross there were 38 cross-overs out of 109 or 34.8 per cent. Crossing-over in the F_2 was less, but the F_2 was small and no importance can be attached to the actual value. The F_1 plant 985 was back-crossed to Burma Laciniated, and in a family of 19 plants two had broad leaves. It was suspected that these were the result of mutation, and they were crossed back again to Burma Laciniated. The plant with a mean index of 2.3 gave 62 broad-leaved seedlings, and that

with a mean index of 2.8 gave 61 laciniated and 51 broad-leaved seedlings. The broad leaf of Mutant Broad is dominant (see below). These two plants were therefore Mutant Broad, one homozygous and one heterozygous.

In the cross Burma Laciniated \times N289 simple segregation into laciniated and broad occurred. In F_2 there were:

	Laciniated	Broad	Total
Observed	115	41	156
Expected	117.0	39.0	156.0

and in the back-cross to 1304 there were:

	Laciniated	Broad	Total
Observed	14	15	29
Expected	14.5	14.5	29.0

In the back-cross to Cawnpore White there were laciniated plants, and plants with leaf shape similar to that of heterozygotes between Cawnpore White and N289 (see Table IV).

	Laciniated	Intermediate	Total
Observed	14	14	28
Expected	14.0	14.0	28.0

From a family of ((Cawnpore White \times N289) \times Burma Laciniated) 786 selfed, plant 183 with laciniated leaves was selected and crossed by "Chickenfoot." The F_1 's were grown under the number 31-5. Two plants with mean indices of 10.6 and 8.6 were selected and back-crossed to 1304. They gave offspring with laciniated and *arboreum*-type leaves in equal numbers. They were grown under good conditions, and the leaves were large and well developed. The distinction between the two types was clear enough for eye classification to be made quite easily. A frequency array for the back-crosses of the two F_1 's is given in Table VI. There were in all: 143 *arboreum* : 144 laciniated.

Since the narrow leaf of Cawnpore White is allelomorph to the broad leaf of 1304 and N289 (see Part I), and since laciniated is allelomorph to the narrow of Cawnpore White and the broad of 1304 and N289, laciniated, narrow and broad must form a multiple allelomorph series.

(2) Crosses within the Burma Laciniated strain.

The Mutant Broad and Mutant Intermediate types obtained from Burma Laciniated were crossed on to the parent type and on to each other. Frequency arrays of mean index for the F_1 's are given in Table VII.

In contrast with other Burma Laciniated crosses, broad leaf was dominant over narrow, and the leaves of the F_1 's were quite indistinguishable from those of their broad-leaved parents.

Three F_1 plants of Burma Laciniated \times A9 with mean indices of 2.2 and 2.3 were back-crossed on to Burma Laciniated. Ninety-seven plants were obtained. All had broad leaves like the mutant parent. Mean indices were calculated for 63 typical plants, and are given in Table VII.

The back-cross distribution is practically identical with the F_1 distribution, and very little different from the distribution of the parent Mutant Broad.

Eleven plants were selected from the back-cross and back-crossed again to Burma Laciniated. Five of these were also back-crossed to Cawnpore White. In order to save labour, a part of the back-cross families was searched for laciniated plants in the seedling stage. The rest were grown to maturity and measured. Results are summarised in Table VIII.

Ten out of the 11 plants used for the second back-cross yielded nothing but broad-leaved offspring when crossed either with Burma Laciniated or with Cawnpore White. The frequency distributions are very similar to those of the original Mutant Broad parent.

Plant 764 of the first back-cross gave families in the second back-cross in which approximately one-third of the plants had mean indices greater than 2.8, the highest mean index in the other 10 families.

Six plants of the second back-cross to Burma Laciniated from families giving broad only were back-crossed again to Burma Laciniated and with a single exception gave broad-leaved offspring only, 140 plants in all. In one family one laciniated seedling appeared out of 36. Since Burma Laciniated was the female parent, it is probable that this was an accidental self.

Eleven plants of the back-cross to Cawnpore White from families giving broad only were back-crossed again to Burma Laciniated. Broad and laciniated seedlings appeared. There was a slight but persistent excess of laciniated over the 50 per cent. expected for single-factor segregation. Results are given below.

	9	12	8	6	10	1	3	8	4	9	3	Total
Narrow	9	12	8	6	10	1	3	8	4	9	3	73
Broad	4	10	5	4	9	—	4	8	—	12	1	57
Total	13	22	13	10	19	1	7	16	4	21	4	130
Difference	+5	+2	+3	+2	+1	+1	-1	0	+4	-3	+2	+16

On the total $\chi^2 (1:1) = 1.97$, $n = 1$, $P = 0.15$.

The deviation from the 1:1 cannot, therefore, be judged significant.

Treating the differences by the "t" method (Fisher, 1932) to test the significance of the mean difference from 0 gives:

$$M = 1.4545, t = 2.14, n = 10, \text{ and } P = 0.06.$$

The differences agree together sufficiently, therefore, to increase the value of the deviation to a level where it cannot be disregarded.

The meaning of the deviation will be discussed later under the cross Mutant Broad \times Cawnpore White.

To sum up: the laciniated character cannot be recovered from a cross of Burma Laciniated \times Mutant Broad by back-crossing to Burma Laciniated. With the exception of plant 764, each generation behaves as if homozygous for Mutant Broad. On the other hand, in crosses of Mutant Broad \times Cawnpore White, all plants behaved as heterozygotes.

Two progenies were obtained from plant 764, a back-cross of 110 plants with Cawnpore White as male parent and a back-cross of 63 plants with Burma Laciniated as male parent. Leaf measurements were taken before the plants began to flower. Frequency arrays of mean index are given in Table VIII. Both arrays fall into two groups, with a natural division at 2.7. Dividing at 2.7 gives:

	Broad	Intermediate and narrow	Total
764 \times C.W.	70	40	110
764 \times B.L.	42	21	63

The intermediate class in the back-cross to Cawnpore White is similar in distribution to the narrow class in (Cawnpore White \times N289) \times N289, but somewhat lower (see Table IV). It resembles therefore the heterozygote (Ll) between *arboreum* and recessive broad. The intermediate and narrow class in the back-cross to Burma Laciniated was made up of laciniated and intermediate. Mutation was observed in the back-cross to Burma Laciniated, and accordingly leaf measurements were again taken after the plants had flowered. In Table XI is given a correlation table of mean index at first and at second measurement.

Only half of the back-cross to Burma Laciniated was available for a second measurement. All the plants which originally had mean indices between 3.0 and 4.0, and which were available for measurement a second time, had become broader leaved, and gave mean indices from 2.4 to 2.8. The plants for which two leaf measurements are available fall into three groups: (1) 17 plants which had mean indices below 2.5 at both measurements; (2) nine plants with mean indices above 2.5 at one or other or both measurements, and not above 4.0 at either; and (3) six plants with mean indices 4.0 or higher at both measurements. The plants which mutated between the first and second measurements were all in the second class. The plants only measured once can be divided correspondingly into 20 group (1) : 5 group (2) : 6 group (3), giving in all 37 : 14 : 12.

The back-cross to Cawnpore White was relatively stable, but there was strong evidence that mutation had taken place in a few plants. One plant dropped in mean index from 3.1 to 2.3 between the first and second measurements, and another from 2.9 to 2.5.

Owing to lack of time and space it was not possible to grow progenies from both back-crosses. The back-cross to Cawnpore White was discarded, and selfed seed obtained from the back-cross to Burma Laciniated. Families were grown from 38 plants, and leaf measurements were taken. Summary frequency arrays are given in Table VIII. All plants with mean indices from 2.1 to 2.4 gave similar distributions with no plants having mean indices above 2.8. These correspond to the plants in group (1) on the correlation table (Table XI) of first and second measurements. Plants in group (2) (mean indices from 2.5 to 4.0 at the first measurement) gave some plants with mean indices of 3.0 and upwards. Plants 36, 37 and 43 gave also a very small proportion of laciniated offspring. These were plants in which there was the greatest change in leaf shape between the first and second measurement. A progeny was grown from a second crop of seed from plant 37, and no laciniated plants occurred in a family of 153. Plants in group (3) (laciniated at both measurements) gave laciniated, intermediate and broad-leaved offspring. In the progenies of plants 33, 35 and 40, only a few intermediates occurred. In the progeny of plant 39 there were many. Dividing into broad and (laciniated + intermediate) gives:

Family	Broad	Laciniated + intermediate	Total
33	4	16	20
35	18	53	71
39	21	80	101
40	6	16	22
Total	49	165	214
Expected (1 : 3)	53.5	160.5	214.0

The four families gave a close approach to 3 narrow : 1 broad, and must therefore have been heterozygotes between laciniated and recessive broad. The intermediates were mosaics of mutant and non-mutant tissue. The behaviour of such mosaics will be described later.

Six broad-leaved segregates from plant 39 and three from plant 35 were crossed by an *arboreum*. In every case the progenies had leaves with mean indices from 2.8 to 4.3, typical of heterozygotes between *arboreum* and recessive broad. One broad-leaved segregate from plant 39 was crossed by Burma Laciniated, and gave laciniated-leaved offspring only, with mean indices from 4.8 to 8.5. When it was discovered that the laciniated plants were behaving as L^{L1} heterozygotes, they were back-

crossed to *G. herbaceum* (recessive broad). The back-cross families were classified in the seedling stage into laciniated and broad. Results are given below.

Family	Broad	Intermediate	Laciniated	Total
<i>herbaceum</i> × 33	100	5	—	105
<i>herbaceum</i> × 35	23	—	21	44
<i>herbaceum</i> × 39	84	—	83	167
<i>herbaceum</i> × 42	14	—	14	28
<i>herbaceum</i> × 44	26	—	16	42

The plants were cut back after selfed seed was obtained, and all young branches on plant 33 had broad leaves. Evidently about 95 per cent. of eggs and pollen grains carried L^B or l . Plant 44 subsequently underwent mutation, and the ratio of 26 broad : 16 laciniated suggests that mutant tissue was already present when the back-cross was made. In progenies of plants 35, 39 and 42, the normal gametic ratio of $1L^L : 1l$ was obtained.

Plant 39 later mutated to broad leaf. A cross of the mutant 39 by 24-2 gave 14 plants with mean indices from 3.2 to 3.8, typical of Ll heterozygotes, so it must have mutated to homozygous recessive broad, ll .

Seven plants of groups (1) and (2) were crossed to Burma Laciniated or the *arboreum* type 24-2. Mean index frequency arrays are given in Table X. Of these plants 13 and 27 were from group (1), with mean indices below 2.5 at both measurements. Crossed to Burma Laciniated, plant 13 gave 160 broad-leaved plants only. Leaf measurements were taken on 63 plants, and gave mean indices from 1.9 to 2.5. Plant 27 × Burma Laciniated gave 98 broad-leaved plants. Leaf measurements were taken on 53 plants. Of these 52 had mean indices from 2.0 to 2.4, and one had a mean index of 2.8. Plant 27 was also crossed to 24-2, and gave a family of 30 plants with mean indices from 2.4 to 2.9. These two plants were evidently homozygous $L^B L^B$.

Five plants, 21, 25, 36, 41 and 43 were tested which belonged to group (2) and gave on selfing families with mean indices ranging from 2.2 to 3.2.

Plant 21 was crossed by Burma Laciniated, and out of 156 plants, 79 were intermediate broad, with mean indices from 2.3 to 3.2, and 77 were narrow or laciniated, with mean indices from 3.3 to 7.7. Classification by eye was easy in the field. Narrow-leaved segregates from 21 × Burma Laciniated were assumed to be mosaics of $L^L l$ and $L^L 1$ or $L^B 1$ tissue. The plants were therefore examined carefully for evidence of unequal distribution of mutant and normal tissue. Plants were found with entirely broad-leaved branches, or with a sector of the stem giving rise to a series of

leaves broader than the rest, or with odd leaves with broad lobes on one side and narrow lobes on the other. A frequency array is given in Table X of plants on which visible differences in leaf shape occurred. Of 19 plants with mean indices between 3.3 and 4.3, 12 were visibly mosaics, and of 58 plants with mean indices above 4.4, only three showed any sign of mutant tissue.

Plant 21 was also crossed on to 24-2, and gave 63 plants of which 34 were intermediate broad leaved, with mean indices from 2.6 to 3.1, and 27 were narrow leaved, with mean indices from 3.3 to 4.1.

Plant 25 was crossed by Burma Lacinated. Nineteen plants were obtained, of which 12 were intermediate broad leaved, with mean indices from 2.6 to 3.2, and seven were narrow or lacinated, with mean indices from 3.8 to 5.4. Two plants with mean indices of 4.3 and 4.7 were visibly chimaeras.

Plants 21 and 25 appear to have been heterozygotes between mutant intermediate and recessive broad.

Plant 41 was crossed by 24-2 and gave eight plants with mean indices from 3.2 to 3.8. Since on selfing it gave plants with mean indices from 2.3 to 3.2, it must have been homozygous recessive broad, **11**.

Plants 36 and 43 gave on selfing progenies with mean indices ranging from 2.2 to 3.4, with occasional plants with lacinated leaves. Both were crossed by 24-2. Plant 36 gave 16 plants with mean indices from 3.1 to 4.0, and plant 43 gave 35 plants with mean indices from 3.2 to 4.3. These two plants must therefore have become homozygous recessive broad **11**.

Of the plants tested, both from group (1) proved to be homozygous mutant broad, and all from groups (2) and (3) to be either heterozygous or homozygous recessive broad.

Taking groups (2) and (3) as including plants carrying **1** in the cross of 764 \times Burma Lacinated, and plants with mean indices of 2.7 and upwards at the first measurement as carrying **1** in the cross of 764 \times Cawnpore White, gives the following estimates of the gametic ratio in plant 764:

764 \times B.L.	37 L^B : 26 1
764 \times C.W.	70 L^B : 40 1 .

These agree well, and combined give

$$0.62 \mathbf{L^B} : 0.38 \mathbf{1}.$$

Plant 764 must originally have been homozygous **L^BL^B**, and have become by mutation a mosaic of **L^B1** and **L^BL^B** tissue, or **L^BL^B** and **11** tissue.

Most of the families grown from plants of 764 \times Burma Laciniated with mean indices from 2.1 to 2.4 were pulled up as soon as leaf measurements had been taken. Families with intermediate or narrow-leaved plants were left and watched for mutation. When the bolls began to open, it was found that some of these families were segregating for lint colour. Three families from plants in group (1) giving broad-leaved offspring only were left long enough to be classified for lint colour. Two of these proved to be homozygous brown lint. Plant 27 gave one brown-linted plant and two white-linted plants. Progenies of 10 plants of group (2) (mean indices from 2.5 to 4.0 at first measurement) were classified for lint colour. Of these three plants proved to be homozygous for brown lint, and seven gave both brown-linted and white-linted progeny. Frequency arrays for mean index are given separately for the lint-colour classes in Table IX. In all cases where there are enough results to make comparison possible, brown-linted segregates had on the average higher mean indices than white-linted segregates. Of the four group (3) families available, three were homozygous for brown lint. In the progeny of plant 35, brown-linted and white-linted segregates occurred. Frequency arrays are given in Table IX. Summarising in the four classes there were:

	Laciniated		Broad		
	Brown	White	Brown	White	Total
Observed	39	9	10	6	64
Expected (33% c.o.)	39	9	9	7	64

Plant 35 was crossed by a white-linted broad-leaved plant from among its progeny. In a family of 243 plants there were:

Laciniated		Broad		
Brown	White	Brown	White	Total
82	31	36	94	243

The results from the selfed progeny fit expectation with 33 per cent. crossing-over, and those of the back-cross family, 27.6 per cent. crossing-over, agreeing well with the results from Burma Laciniated \times Cawnpore White and Burma Laciniated \times 1304.

Mean index frequency-arrays for F_2 and back-cross of Mutant Intermediate \times Burma Laciniated are given in Table VII. In F_2 (progeny of two F_1 plants) and in a back-cross to Mutant Intermediate, the leaf shape of all plants was indistinguishable from that of Mutant Intermediate, and the frequency arrays of mean index in F_2 and $F_1 \times$ Mutant Intermediate are very similar to that of Mutant Intermediate. In the back-cross to Million Dollar, the frequency array runs lower than

that of Mutant Intermediate, no doubt as the result of the effect of Million Dollar genes. The F_1 was also back-crossed to Burma Laciniated. The back-cross was classified in the seedling stage and gave 125 broad-leaved seedlings only.

In F_2 (progeny of three F_1 plants) and back-crosses to recessive broad of Mutant Intermediate \times Mutant Broad, all plants had leaves like Mutant Broad, and mean index frequency-arrays given in Table VII are very similar to those of the F_1 (Table VII) and of Mutant Broad (Table I).

A back-cross to Burma Laciniated gave 56 broad-leaved seedlings only.

The results of crosses within the Burma Laciniated strain may be summarised as follows:

(1) Heterozygotes between Burma Laciniated and Mutant Broad mutate from $L^L L^B$ to $L^B L^B$.

(2) Heterozygotes between Burma Laciniated and Mutant Intermediate mutate from $L^L L^I$ to $L^I L^I$.

(3) The L^B gene of Mutant Broad is also somewhat unstable, and on one occasion mutated to l , the brown-lint gene K at the same time mutating to k . Since the cross-over rate is unchanged in mutant stocks the change must be due to two point mutations and not to loss of a section of a chromosome.

(4) Heterozygotes between Burma Laciniated and recessive broad derived from it by mutation are unstable, but not so unstable as $L^L L^B$ and $L^L L^I$ heterozygotes. They may:

- | | | | | | |
|--|-----|-----|-----|-----|---------|
| (a) Remain unchanged | ... | ... | ... | ... | $L^L l$ |
| (b) Mutate to homozygous recessive broad | ... | ... | ... | ... | ll |
| (c) Mutate to heterozygous intermediate mutant | ... | ... | ... | ... | $L^L l$ |

Mutation to $L^I l$ seems to be completed more rapidly than to ll , since two of the three plants from the back-cross of 764 \times Burma Laciniated which proved to be ll gave a few laciniated offspring among their first progeny, and were among those which changed most in leaf shape between the first and second measurement, whereas the two plants shown to be $L^I l$ were intermediate broad from the seedling stage and never gave any laciniated leaved offspring on selfing. Mutation from $L^L l$ to $L^B l$ may possibly occur, but the close agreement between the proportions of narrow-leaved plants in the back-crosses of 764 \times Burma Laciniated and 764 \times Cawnpore White makes it unlikely that $L^B l$ plants occurred in the former.

The occurrence of one plant in the cross 27 \times Burma Laciniated with a mean index of 2.8 suggests that an l gamete occurred by mutation from

L^B , and that on fertilisation with an L^L gamete, mutation occurred to heterozygous intermediate mutant.

Three similar plants occurred in the original F_1 of $A9 \times$ Burma Laciniated, but at that time the importance of so small a difference in leaf shape was not realised, and their constitution was not investigated.

(3) *Crosses of mutants with unrelated types.*

Mutants \times Cawnpore White.

Both mutants were crossed by Cawnpore White. The F_1 's resembled the mutant parents closely.

Back-crosses of Mutant Broad \times Cawnpore White were made to Cawnpore White, 1304, and Burma Laciniated. Owing to unfavourable weather conditions, not all plants grew well enough for leaf-index determinations to be made. In the back-cross of ($A9 \times$ Cawnpore White) \times Cawnpore White and ($A9 \times$ Cawnpore White) \times 1304 it was possible to classify the plants into narrow and broad by eye. The classification was checked by two independent observers, and was later checked against leaf-index determinations for those plants on which they were taken. Satisfactory agreement was obtained in all cases. Leaf-index and lint-colour results are given in Table XII.

The mean index distribution for the back-cross to Cawnpore White falls into two groups with a division at 3.0-3.3. The distribution of mean index in the broad class is similar to that of the F_1 , and in the narrow class is similar to that of Cawnpore White (Table I). Summing the results from the two F_1 's gave 30 broad : 41 narrow, and 31 brown : 25 white,

Broad		Narrow		Total
Brown	White	Brown	White	
21	5	10	20	56

Cross-over classes amounted to 15 plants out of 56, or 26.8 per cent.

The frequency arrays for the back-cross to 1304 are lower in their range. A natural division occurs at mean index 2.5-2.7, and dividing at 2.7 gave good agreement with the eye classification into narrow and broad. The distribution of the broad class agrees with that of the F_1 of $A9 \times$ 1304 (see Table XIII). The distribution of the narrow-leaved class is considerably lower than would be expected for heterozygotes between *arboreum* and *Nanking*. The difference is ascribed to the very dry conditions under which the plants grew. The leaves were very short, and the sinus length and lobe width were not correspondingly reduced. Classifica-

tion by eye was made possible by the fact that leaves too small and immature for measurement fell more distinctly into their respective classes than fully grown leaves on which the effect of stunting was greater. Summing the results for the two F_1 's gave 77 brown : 79 white-linted plants, or very nearly equal numbers. There were 110 broad : 82 narrow-leaved plants, a significant excess of broad-leaved plants.

Broad		Narrow		Total
Brown	White	Brown	White	
59	32	18	47	156

Cross-overs amounted to 50 out of 156, or 32 per cent.

In the back-cross to Burma Laciniated leaf-index measurements were taken on all plants, but the mean indices of laciniated-leaved plants were in many cases considerably lower than would be expected owing to the adverse effect of dry conditions. In the back-crosses from the two F_1 's there were 21 broad : 30 laciniated.

The proportion of L^B gametes formed by the F_1 may be estimated from the back-cross to Cawnpore White and to Burma Laciniated. There were:

Back-cross	Broad	Narrow	Total
$F_1 \times C.W.$	30	41	71
$F_1 \times B.L.$	21	30	51
Total	51	71	122

$\chi^2 (1 : 1) = 3.28$, $P = 0.07$. The difference as it stands can hardly be regarded as significant.

To these results may be added the results given above from ((Burma Laciniated \times A9) \times Burma Laciniated) \times Cawnpore White) \times Burma Laciniated, which gave on seedling counts of 11 families:

Broad	Narrow	Total
57	73	130

In back-crosses of this type there were obtained in all, therefore,

Broad	Narrow	Total
108	144	252

$\chi^2 (1 : 1) = 5.1$, $n = 1$, $P =$ between 0.02 and 0.01.

On the total results, therefore, the excess of narrow-leaved types is undoubtedly significant. The excess may be ascribed to mutation from L^B to l , giving recessive broad gametes which give narrow or laciniated-leaved plants with L or L^L gametes. In Mutant Broad \times Cawnpore White there was no excess of white-linted segregates, which suggests that mutation from L^B to l was not associated in this case with mutation in the brown-lint gene. An estimate of the proportion of L genes formed by the

F_1 is given by the back-cross to 1304. In this case there is undoubtedly an excess of broad-leaved plants, which must be the result of mutation from **L** to **l**.

F_2 's and back-crosses to Cawnpore White were grown of the cross Mutant Intermediate \times Cawnpore White. Mean index frequency-arrays are given in Table XII. F_1 plant 3 gave in F_2 :

Observed Expected (35% c.o.)	Intermediate broad		Narrow		Total
	Brown	White	Brown	White	
	40	11	8	7	66
	40.0	9.5	9.5	7.0	66.0

The ratios of broad : narrow and brown : white approach the expected 3 : 1 closely. The proportion of cross-overs indicates a cross-over rate of 35 per cent.

F_1 plant 10 gave an F_2 of 55 plants, all of which had leaves like those of Mutant Intermediate. The *arboreum* class was not recovered. In this F_2 there were 37 brown : 18 white-linted plants, showing that normal segregation occurred for lint colour.

In the back-cross to Cawnpore White, both F_1 plants behaved as if heterozygous for leaf shape.

	Broad		Narrow		Total
	Brown	White	Brown	White	
F_1 3 \times C.W.	26	12	13	27	78
F_1 10 \times C.W.	7	4	5	6	22

Both F_1 's gave approximately equal numbers for both segregating genes. Cross-over classes amounted to 34 plants out of 100.

In F_1 plant 10, mutation must have occurred from **L¹L** to **L¹l**, so that in F_2 only intermediate mutant and recessive broad segregates were obtained, while in a back-cross to Cawnpore White intermediate mutant and heterozygous (**Ll**) *arboreum* segregates were obtained.

Mutant Broad \times Bengal A13.

Bengal A13 is a typical *arboreum* with a leaf closely similar to that of Cawnpore White. It lacks the brown-lint gene (**K**), but carries independent genes for grey lint. Two F_1 plants had mean indices of 2.7. The first flowers on each F_1 plant were emasculated and pollinated with Cawnpore White pollen. Later flowers were selfed. F_2 's and the back-crosses from both F_1 's were planted out. The F_2 from F_1 plant 2 segregated into approximately 3 broad-leaved : 1 narrow-leaved offspring, but unfortunately it was discovered that seed from an unrelated type had been mixed in, and the F_2 was accordingly destroyed. There remained some

selfed seed from the last bolls on the plant, and this was planted out late in the season. The plants did not grow well, but it was possible to classify them into broad and *arboreum* segregates. In a family of 229 plants there were:

222 broad : 7 *arboreum*.

The F_2 from F_1 plant 1 and both back-crosses grew well, and leaf measurements were taken. Mean index frequency-arrays are given separately for the lint-colour classes in Table XII. In the back-crosses there were approximately equal numbers of narrow and broad-leaved plants, and of brown and (grey and white) linted plants, with brown lint coupled with broad leaf:

	Broad		Narrow		Total.
	Brown	Grey and white	Brown	Grey and white	
$F_1 1 \times \text{C.W.}$ Observed	48	20	16	54	138
$F_1 2 \times \text{C.W.}$ Observed	22	6	11	18	57
Total Observed	70	26	27	72	195
Expected (27.2 % c.o.)	71.0	26.5	26.5	71.0	195.0

There was no excess of narrow-leaved plants, such as occurred in the back-crosses of Mutant Broad \times Cawnpore White to Cawnpore White and to Burma Laciniated.

Except for two plants with mean indices of 3.1 and 3.2 in the back-cross of $F_1 1 \times$ Cawnpore White, the distribution of the narrow-leaved segregates agrees well with that of homozygous *arboreums* (see Table I).

In the F_2 from F_1 plant 1 there were only seven plants out of 78 with *arboreum*-type leaves. There were in addition a number of plants with mean indices from about 3.0 to 3.4, the leaves on which resembled those of *arboreum* \times recessive broad heterozygotes. The effects of linkage between **K** and leaf shape have disappeared. Plants with leaves resembling those of **Ll** heterozygotes were also observed in the later F_2 from F_1 plant 2. Evidently mutation occurred in the F_1 's from **L^BL** to **L^Bl**.

Consideration of the order in which the seed which gave rise to the different families was produced shows that the mutation was progressive.

(1) The first flowers on F_1 plant 2 gave rise to **L^B** and **L** gametes only giving in the back-cross to Cawnpore White **LL** and **L^BL** plants only, and in the early F_2 approximately 25 per cent. of **LL** plants.

(2) The first flowers on F_1 plant 1 gave a small proportion of **l** gametes in addition to **L^B** and **L**, as shown by the occurrence of two intermediate (**Ll**) plants in the back-cross.

(3) Mutation proceeded more rapidly than in F_1 plant 2, since there

were only seven **LL** plants out of 78 and a considerable number of **Ll** plants in the F_2 from fairly early seed.

(4) Mutation finally occurred in F_1 plant 2, and the F_2 from late seed gave only seven **LL** plants out of 229.

Leaf measurements were taken on the F_2 from F_1 plant 1 as soon as the plants began to flower. About six weeks later leaf measurements were again taken on the youngest leaves on the main stem in order to see whether somatic mutation had occurred. There was no evidence of mutation, and the mean indices at the first and the second measurement agreed well together. Somatic mutation to **L^Bl** in **L^BL** heterozygotes would, of course, not be visible, since **L^B** is dominant.

Mutants \times recessive broad.

985 Mutant. This arose as a broad-leaved branch on an F_1 plant of Burma Laciniated \times 1304. Its mean index was 2.5, and it was indistinguishable from a mutant broad. An F_2 and back-crosses to 1304, Burma Laciniated, and Cawnpore White were grown. Frequency arrays of mean index are given in Table XIII. In F_2 and in the back-cross to 1304 all plants had broad leaves. In the back-cross to Cawnpore White, two classes appeared, broad like mutant broad, and narrow similar to heterozygotes between *arboreum* and *Nanking* (see Table III). Segregation also occurred for lint colour. Dividing at 3.0 gives:

	Broad		Narrow		Total
	Brown	White	Brown	White	
Observed	7	1	5	4	17
Expected (36 % c.o.)	5.5	3.0	3.0	5.5	17.0

In the back-cross to Burma Laciniated also, two classes appeared, broad like mutant broad, and laciniated:

	Broad	Laciniated	Total
Observed	16	17	33
Expected	16.5	16.5	33.0

One exceptional plant occurred, plant 254, which had a mean index of 3.3. Five of the broad-leaved segregates were crossed on to Burma Laciniated, and gave 205 broad-leaved plants and no laciniated.

985 Mutant therefore behaved as a simple heterozygote between Mutant Broad (dominant) and the recessive broad of *G. arboreum* var. *Nanking*. **L^B** genes from 985 Mutant had the same effect in inducing mutation in **L^L** genes as had **L^B** genes from the original Mutant Broad strain.

The origin of two broad-leaved plants in the back-cross of (Burma Laciniated \times 1304) \times Burma Laciniated reported above (p 456) will now

be clear. They were fresh mutants analogous to 985 Mutant. One in which the original zygotic constitution must have been $L^L L^L$ mutated to homozygous broad. The other, in which the zygotic constitution must have been $L^L l$, mutated to $L^B l$.

Mutant broad \times 1304.

The F_1 had a mean index distribution similar to that of Mutant Broad (Table XIII). Back-crosses to 1304, Cawnpore White and Burma Laciniated were made on two F_1 plants. Dry weather interfered with the growth of these families. In the back-cross to Cawnpore White classification by eye was resorted to and checked against leaf measurements on the best plants. In the back-cross to Burma Laciniated all plants were measured, and the distribution of the laciniated class is accordingly a good deal lower than would have been the case if conditions had been favourable. Since adverse conditions have little effect on the mean index of broad-leaved types, the distribution of the back-cross to 1304 was fairly typical.

The back-crosses to Cawnpore White may be divided at 3.0, giving:

	Broad		Narrow		Total
	Brown	White	Brown	White	
Observed	70	40	29	70	209
Expected (33 % c.o.)	70	34.5	34.5	70	209

There were 99 brown : 110 white-linted plants, and 110 broad : 99 narrow-leaved plants, or approximately equal numbers in each case. The cross-over rate was 33 per cent.

The back-cross to 1304 gave broad-leaved plants only, and 52 brown : 48 white-linted plants.

The back-cross to Burma Laciniated fell into two groups for leaf shape, narrow and broad.

	Broad	Narrow	Total
$F_1 1 \times B.L.$	88	25	113
$F_1 4 \times B.L.$	62	36	98

Among the narrows were a number with mean indices between 3.1 and 4.0. Some of these were no doubt stunted laciniated plants, but some were similar to plant 254 in the back-cross of 985 Mutant \times Burma Laciniated. In the broad class there were true mutant broad types, and types similar to mutant intermediate with mean indices from about 2.5 to 3.1. That the deficiency of laciniated is not due to a deficiency of 1 gametes is shown by the fact that the ratio of broad : narrow in the back-cross to Cawnpore White is not disturbed, and it may be concluded that mutation occurred in a considerable proportion of the young plants from $L^L l$ to $L^I l$, and possibly $L^B l$.

The occurrence of plants with mean indices similar to those of *arboreum* types, where laciniated and broad-leaved segregates only were expected, has been noted in the progeny of L^L1 heterozygotes from $764 \times$ Burma Laciniated, (Mutant Broad \times Cawnpore White) \times Burma Laciniated, (Mutant Broad \times 1304) \times Burma Laciniated, and in $(764 \times$ Burma Laciniated) $21 \times$ Burma Laciniated. On examination such plants usually showed clear signs of mutation. Occasionally there were some whole branches bearing laciniated leaves, and others bearing only broad leaves. In other cases the only evidence of mutation was that parts of some leaves were laciniated. In a large F_1 family of Burma Laciniated \times 1304 several definite chimaeras were discovered. An outline drawing of a branch of one of these, showing a streak of L^L tissue persisting in an otherwise mutant branch, is reproduced in Fig. 19.

Plant 254 from the back-cross of 985 Mutant \times Burma Laciniated was taken as typical of these plants with *arboreum* leaf shape and was tested to see what genes were present. As soon as its abnormal leaf shape was observed, flowers were selfed, and selfed seed obtained. The plant was examined carefully, and a single monopodial branch near the base was found which bore laciniated leaves. This branch, and the next monopodium above it, were grafted, and labelled 254A and 254B respectively. A third graft was taken from a branch near the top of the plant with a leaf shape typical of the greater part of the plant. This was labelled 254C.

When the grafts were well grown leaf measurements were taken, and gave the following results:

Graft	Mean index
A	3.9
B	3.8
C	2.7

Grafts A and B had narrower leaves than had 254 when originally measured, and graft C had broader leaves. All three grafts were crossed by Burma Laciniated, and by two recessive broads, 1304 and Million Dollar. When sufficient crosses had been made, the subsequent flowers were left to provide selfed seed. It was observed that the distal portions of the two grafts 254A and 254B had leaves as broad as those of 254C, and measurements taken on the later leaves of the lateral monopodia gave the following mean indices:

Graft	Mean index
254A	2.8
254B	2.7

The original selfed seed from plant 254 was planted out in the green-

house. The crosses and selfed seed from the grafts were planted in the field. Leaf measurements were taken and indices calculated. Results are given in Table XIV.

A number of plants with mean indices about what would be expected for *L* (*arboreum*) occurred in most progenies. These are classed as laciniated. Examination in the field in several instances showed that true laciniated leaves occurred on parts of these plants.

The selfed seed of the original plant 254 gave 69 plants. Of these 40, or 58 per cent., were laciniated, with mean indices from 4.0 to 7.0 and 28, or 41 per cent., were intermediate broad, with mean indices from 2.4 to 3.1. One plant had a mean index of 3.4.

Graft C, which was taken from a typical branch of plant 254, gave on selfing and on crossing to 1304 nothing but intermediate broad-leaved plants, and on crossing to Burma Laciniated gave 15 intermediate broads, two with mean indices of 3.4 and eight laciniated.

The selfed seed from grafts A and B also gave broad-leaved plants only. Both grafts were pollinated with 1304 and with Million Dollar, and in the resulting families intermediate broad and laciniated were obtained.

	Intermediate broad	Laciniated	Total
254A ♀ × M.D.	14	6	20
254A ♀ × 1304	20	1	21
254B ♀ × M.D.	26	5	31
254B ♀ × 1304	9	3	12
Total	69	15	84

The proportion of laciniated plants obtained varied considerably, but in all cases there was a large preponderance of intermediate broads.

One small family was obtained from the cross 254B ♀ × Burma Laciniated ♂ and larger families of 254A ♂ × Burma Laciniated ♀ and 254B ♂ × Burma Laciniated ♀. Laciniated and intermediate broad were again obtained.

	Intermediate broad	Laciniated	Total
254A ♂ × B.L.	10	31	41
254B ♂ × B.L.	11	67	78
254B ♀ × B.L.	20	13	33

Where 254 pollen was used, a large preponderance of laciniated offspring was obtained. Where 254 eggs were used, intermediate broad offspring were in excess.

There is no evidence of the presence of *L* genes. The narrow-leaved segregates in the back-crosses to recessive broad were either laciniated or mutant types similar to the original plant 254. The results can, however, be explained on the assumption that the original 254 zygote was *L*^L1, and

as the plant developed, mutation occurred, giving rise to L^1 tissue. The *arboresum* leaf shape resulted from the mixture of the two tissues to form an unstable mosaic. In graft C, L^1 tissue had entirely disappeared, leaving a homogeneous L^1 heterozygote.

The failure to obtain laciniated plants from 254 A and 254 B by selfing was ascribed to further mutation in the parts of the plants from which selfed seed was obtained, as a result of which all L^1 genes disappeared, and the leaves became intermediate broad. The two plants were cut back and branches arose from near the base which bore leaves with mean indices of about 3.4. It was hoped that these branches would carry the gene L^1 and laciniated plants would be obtained on selfing. Selfed seed was obtained from them, and also back-crosses to 1304. Mean index distributions for their progeny are given in Table XIV.

Some families were classified in the seedling stage into narrow and broad, and were not grown on in the field. Results for all families are summarised below.

Family	Broad	Narrow	Total
254A selfed	63	1	64
1304 - 34 ♀ × 254A ♂	130	18	148
254B selfed	48	1	49
1304 - 34 ♀ × 254B ♂	154	7	161

In 254A selfed one plant out of 64 had a mean index of 3.7. All others were broad leaved. In the cross to 1304, 18 plants out of 148, or 12 per cent., were classified as narrow. Of these, leaf indices are available for seven, and they varied from 3.5 to 5.9.

In 254B selfed one plant out of 49 had a mean index of 3.4. In the cross to 1304, only seven plants out of 161, or 4 per cent., were classified as narrow, and these had mean indices from 3.6 to 5.6.

The attempt to obtain laciniated plants by selfing failed, and only 12 per cent. of 254 A pollen and 4 per cent. of 254 B pollen appears to have carried L^1 . Most of the plants carrying L^1 had leaves similar to those of the original 254, and were probably mosaics.

The instability of plants composed of tissues of different genotypes is shown by the rapidity with which grafts A and B of plant 254, and narrow-leaved plants in the back-cross of 764 × Burma Laciniated became broad-leaved. From the reciprocal back-crosses of 254 B × Burma Laciniated it appears that L^1 gametes disappear from among eggs more rapidly than from among pollen grains.

814 Mutant. The origin of this mutant is given above. The mutant branches grafted varied in leaf shape (see Table XIII), but after they had grown considerably, they resembled each other closely. Later experience

suggests that they were originally a series of mosaics similar to plant 254 (above) and became homogeneous by further mutation. No differences in behaviour were observed in their progeny, and results from the progeny of all mutant branches have accordingly been lumped together.

Frequency arrays are given in Table XIII for an F_2 from a laciniated branch, and from mutant branches, and a cross of mutant branches to Burma Laciniated.

The laciniated branch gave in F_2 laciniated and broad in the proportions expected for monofactorial inheritance with broad recessive:

	Broad	Laciniated	Total
Observed	9	32	41
Expected	10.25	30.75	41.00

814 Mutant gave in F_2 a frequency array covering the range of recessive broad and Mutant Intermediate. There is a suggestion of a division at 2.4, which corresponds fairly well with the upper limit of the recessive broad class in the 814 laciniated F_2 and approximately one-quarter of the array lies in the range 2.0-2.4.

In the back-cross of 814 Mutant \times Burma Laciniated, intermediate and laciniated occurred in equal numbers:

	Intermediate	Laciniated	Total
Observed	19	19	38
Expected	19.0	19.0	38.0

The original 814 zygote must have been L^1l , and it became by mutation L^1l .

Mutant Intermediate \times 1304.

The F_1 had a mean index of 2.8 similar to that of Mutant Intermediate (see Table XIII). An F_2 was grown, and a back-cross to Cawn-pore White. Separation of classes was impossible in both cases. Mean index distributions are given in Table XIII. In F_2 white-linted plants had on the average lower mean indices than brown-linted plants, as would be expected with white lint coupled with recessive broad leaf shape. In the back-cross, on the other hand, the mean index distribution of white-linted plants was on the whole higher than that of brown-linted plants, since Ll heterozygotes have on the whole rather higher mean indices than have L^1L and L^1L^1 types.

A back-cross was made to Burma Laciniated, and classified into laciniated (L^1l) and broad L^1L^1 in the seedling stage.

There were:

Laciniated L^1l	Broad L^1L^1	Total
80	84	164

L^1 and l gametes were, therefore, formed in equal numbers by the F_1 .

(4) *The constitution of cernuum.*

Cernuum × Burma Lacinated, $L \times L^L$.

An F_1 of 31 plants was grown. Twenty-seven bore lacinated leaves, and four had more or less broad leaves. A frequency array is given in Table XV. Two plants were selected as parents of F_2 's and back-crosses, one lacinated (plant 801) with a mean index of 5.9, and one intermediate broad, with a mean index of 3.1 (plant 798). The behaviour of plant 798 will be considered with the cross *cernuum* × Mutant Intermediate. F_2 's were grown, and back-crosses to Burma Lacinated, a white-linted *arboreum* (A15), *cernuum* and 1304. Frequency arrays of mean index are given in Table XV.

F_1 plant 801 behaved as a heterozygote between lacinated and *cernuum*, and that *cernuum* leaf shape is controlled by a gene in the leaf-shape multiple allelomorph series is shown by the linkage between leaf shape and lint colour. Below are summarised results from the progeny of plant 801, together with expectation on the assumption of 27 per cent. crossing-over, the amount calculated from the sum of the back-crosses to A15 and to *cernuum*.

Cross		Narrow		Lacinated		Total
		Brown	White	Brown	White	
801 selfed	Observed	5	8	17	6	36
	Expected	4.25	4.75	22.75	4.25	36.0
801 × B.L.	Observed	—	—	42	—	42
	Expected	—	—	42.0	—	42.0
801 × A 15	Observed	10	21	20	8	59
	Expected	8.0	21.5	21.5	8.0	59.0
891 × G.C. 450	Observed	9	25	30	8	72
	Expected	9.75	26.25	26.25	9.75	72.0
801 × 1304	Observed	6	8	4	4	22
	Expected	3.0	8.0	8.0	3.0	22.0

In the F_2 there was an excess of narrow-leaved plants and white-linted plants, but in view of the small size of the family no significance can be attached to the deviations. In the back-crosses to A15 and to *cernuum*, expectation was closely realised, there being in all 65 narrow : 66 lacinated plants, and 69 brown : 62 white-linted plants.

Cernuum × *arboreum*, $L \times L$.

Results are available for two crosses of this type, and mean index frequency arrays for F_1 's, F_2 's and a back-cross are given in Table XV.

The cross *cernuum* 450 × Cawnpore White gave a frequency array in F_2 which transgressed the parental arrays at the higher end but did not give any indication of segregation for a major gene. In a back-cross of *cernuum* 450 × Cawnpore White to 1304 a frequency array was obtained

similar to what would be expected from *arboresum* \times *Nanking* (Ll) heterozygotes.

When the *cernuum* strain was first obtained from India, a cross was made between one of the original plants and Cawnpore White. Of four F_1 plants, three had mean indices of 5.5 and 5.8, and one had a mean index of 4.4, similar to the mean indices of F_1 's of *cernuum* 450 \times Cawnpore White. An F_2 was grown from the F_1 plant with mean index 5.5. The mean index frequency-array of the F_2 fell into three groups, with gaps at 5.0 and 6.2, and gave approximately 1 : 2 : 1.

3.9-4.8	5.1-6.1	6.5-7.2	Total
11	22	8	41

The distribution of the 3.9-4.8 group corresponds to the upper part of the distribution of *cernuum* 450 \times Cawnpore White F_2 .

Cernuum 450 behaved like an *arboresum*, differing from typical *arboresums* in a few leaf-shape modifying factors. The *cernuum* parent of the early *cernuum* \times Cawnpore White behaved like a heterozygote ($L^L l$) between lacinated and *arboresum*, since the F_1 consisted of three lacinated plants, one of which gave in F_2 $1L^L L^L : 2L^L l : 1ll$, and one *arboresum* plant, similar to *cernuum* 450 \times Cawnpore White F_1 's.

Cernuum \times recessive broad, $L \times l$.

Four crosses of this type were made, and three different types of behaviour were observed. Frequency arrays for mean index are given in Table XVI.

In the cross Million Dollar \times *cernuum* 450, the F_1 distribution was similar to that of the *cernuum* parent. The frequency array of the F_2 and of the back-cross to Million Dollar may be divided at 2.9 and gave:

Family		Broad	Narrow	Total
F_2	Observed	14	49	63
	Expected	15.75	47.25	63.00
$F_1 \times M.D.$	Observed	32	24	56
	Expected	28	28	56

In F_2 there were approximately 3 narrow : 1 broad, and in the back-cross approximately 1 narrow : 1 broad.

In a cross between Million Dollar and *cernuum* 9, out of nine F_1 plants five were broad leaved, with mean indices below 3.0. Two F_2 's were grown, one from F_1 plant 358, with a mean index of 3.9, and one from plant 1 with a mean index of 2.9. Plant 358 gave in F_2 eight plants with mean indices of 3.8-4.9, and five plants with mean indices of 2.8-3.0. Eight F_3 families were grown. Summary frequency arrays are given in Table XVI. Four plants with mean indices from 2.8 to 3.0 bred true to

broad leaf. Two plants with mean indices of 3.8 and 4.0 respectively segregated and gave 18 narrow : 8 broad-leaved plants. Two plants with mean indices of 4.6 gave narrow-leaved progeny only. Plant 358, therefore, behaved as heterozygous **Ll** and only differed from the cross Million Dollar \times *cernuum* 450 in modifying genes which moved both narrow and broad frequency arrays rather higher up the scale. Plant 1 gave in F_2 intermediate broad-leaved plants only.

Similar results were obtained from *cernuum* 9 \times Burma Khaki. Burma Khaki was a broad-leaved brown-linted *Nanking* type. The leaves of the F_1 's were not measured. Two F_2 's were grown. Among the progeny of plant 732 there were 15 narrow : 2 broad-leaved plants. Among the progeny of 728 there were broad-leaved plants only. Sixteen F_3 families were grown and classified for lint colour, and measured for leaf shape. Frequency arrays are given in Table XVII. Only broad and intermediate-leaved types occurred. Segregation occurred for one or more modifying genes. F_2 plants with leaf indices of 2.9 or higher gave no plants with mean leaf indices below 2.5 in F_3 , and F_2 plants with mean indices of 2.4 or lower gave no plants with mean indices above 2.8. That segregation is in independent modifiers and not in a member of the **L** allelomorph series is shown by the absence of association between leaf shape and lint colour.

In a cross of Burma Ghost \times *cernuum* 9, a single F_1 plant had a mean index of 3.1, and in F_2 only intermediate broad plants were obtained.

A cross was made when *cernuum* was first obtained between *cernuum* and 5F. 5F is a Chinese *Nanking* indistinguishable in leaf shape from Million Dollar, and closely related to it. F_2 's were grown from three F_1 's. F_1 plant 409 had a mean index of 4.5. Leaf measurements were not taken from plants 408 and 410. Plants 408 and 410 behaved as **Ll** heterozygotes, and gave in F_2 frequency arrays divisible between 3.1 and 3.2. The point of division is not distinct, and 3.15 was chosen after inspection of the arrays of homozygous broad-leaved F_3 families grown from broad F_2 plants from F_1 plant 409 (see Table XVI). Dividing at 3.15 gave:

	Broad	Narrow	Total
Observed	21	52	73
Expected	18.25	54.75	73.00

F_1 plant 409 behaved as heterozygous **L^Ll**, and gave an F_2 frequency array divisible at 3.2-4.0 giving:

	Broad	Lacinated	Total
Observed	8	23	31
Expected	7.75	23.25	31.00

Nine F_3 families were grown, and measured. Summary frequency arrays

TABLE XVII.

Frequency arrays of mean leaf index in F_3 families of *G. cernuum* 9-63 \times *Burma Khaki* 483-728.

[illegible]

Mean leaf index distributions of certain \times Recessive Broad crosses

Mean leaf index

Mean leaf index distributions for progeny of cernuum \times Mutant Broad, cernuum \times Mutant Intermediate and cernuum \times Burma Lacinated—798.

Mean leaf indexParents

are given in Table XVI. Two broad-leaved plants bred true to broad. Five plants with mean indices of 5.6–7.4 segregated, giving:

	Broad	Lacinated	Total
Observed	21	64	85
Expected	21.25	63.75	85.00

Two plants with mean indices of 4.9 and 6.8 bred true to lacinated. One plant with a mean index of 3.6 occurred in one of the homozygous lacinated families. Later experience suggests that this was a mutant.

The occurrence of an L^L1 heterozygote in the cross of $5F \times cernuum$, and of an L^LL heterozygote in the original cross of $cernuum \times$ Cawnpore White shows that the original *cernuum* strain must have contained L^LL heterozygotes. Whether these were the result of mutation or whether *cernuum* is normally a mixture of types carrying L^L and L could only be ascertained by observations on a fresh lot of *cernuum* from its original habitat. Since the LL strain of *cernuum* was extracted it has consistently bred true, and no broad-leaved mutants have been observed in it. It must, however, be mutable, and mutate to broad under certain circumstances, since of eight *cernuum* \times recessive broad F_1 's tested three have yielded no L segregates in F_2 , and in one F_1 5 plants were broad leaved : 4 narrow.

Cernuum \times mutant broad.

An F_1 plant had a mean index of 2.3. An F_2 was grown, and 48 plants all had mean indices from 2.2 to 2.6. Back-crosses were made to *cernuum* and to an *arboreum* known as A18. Frequency arrays are given in Table XVIII. The back-cross to *cernuum* 450 was examined for plants mosaic for shape, and five plants with mean leaf indices of 2.6, 2.7, 2.8, 2.9 and 3.0 were found on which the leaves varied in shape from *cernuum* type to broad. Dividing the back-cross to *cernuum* between 2.5 and 2.6 gives:

	Broad	Narrow and intermediate	Total
$F_1 \times G.C. 450$	50	36	86

Cross-overs amounted to 22 plants out of 60, or 37 per cent., which fits other results as closely as can be expected in a small family. The back-cross to A18 gave 12 broad : 14 narrow, with no intermediates.

The F_2 results show that mutation had taken place in the F_1 from L^BL to L^B1 or L^BL^B . The back-crosses show that recessive broad gametes were present, so mutation must have been to L^B1 . In view of the instability of *cernuum* L in Ll heterozygotes, as shown by crosses between *cernuum* and recessive broad, the occurrence of intermediates and an excess of broads in the back-cross to *cernuum* was to be expected. The back-cross to A18, though small, shows that L^B and l gametes were formed in equal numbers.

Cernuum × mutant intermediate.

Three F_1 plants had mean indices of 2.5, 2.7 and 2.7 respectively. F_2 's were grown, and back-crosses to Burma Laciniated. Frequency arrays of mean index are given in Table XVIII. In F_2 only intermediates and broads were obtained, and no narrows, no plant having a mean index higher than 3.3. That mutation had occurred from the *cernuum* L gene to broad, is shown by the fact that the frequency array of white-linted plants is on the whole lower than that of brown-linted plants.

In the back-cross to Burma Laciniated, laciniated, narrow and intermediate-leaved plants were obtained. Part of the back-cross was examined carefully in the seedling stage and classified into narrow and broad. The two classes were planted out separately and the narrows were further divided at transplanting into laciniated and mutant. Separate frequency arrays are given in Table XVIII for the narrow and broad classes. The 16 "narrow" seedlings which had mean indices from 2.6 to 3.4 at maturity were those classified as mutant at transplanting. On several of these plants, and on nine of the 14 plants with mean indices from 3.6 to 4.5 leaves of different shapes were observed.

Most of the back-cross of Burma Laciniated was classified into narrow and broad in the seedling stage and then destroyed. There were 463 seedlings classified as broad, and 375 classified as narrow, which agrees as well as can be expected with the results from the part of the back-cross transplanted, which gave 91 broad : 79 narrow. Summing, there were in all 554 broad : 454 narrow at the seedling classification. In view of the instability of the L^1 gene, and the fact that both parents of the F_1 belong to mutable strains, the excess of broad-leaved seedlings was ascribed to mutation in L^1l zygotes. However, in order to make quite certain that the F_1 gave L^1 and l gametes in equal numbers, the plants of the F_2 were crossed again on to Burma Laciniated, and the progeny classified in the seedling stage. Three types of family occurred:

- (1) All broad-leaved seedlings, $L^1L^1 F_2$ plants.
- (2) Approximately equal numbers of narrow and broad-leaved seedlings. $L^1l F_2$ plants.
- (3) All, or nearly all, narrow-leaved seedlings, $ll F_2$ plants.

Among 51 plants tested there were 11 plants which gave in all 346 broad-leaved offspring in the back-cross, 30 plants which gave 385 narrow : 398 broad-leaved offspring, and 10 plants which gave 206 narrow and nine broad-leaved offspring.

Observed	11 L^1L^1	: 30 L^1l	: 10 ll	51
Expected	12.75	25.50	12.75	51.00

or very nearly 1 : 2 : 1.

The behaviour of *cernuum* × Burma Lacinated F_1 plant 798 was similar to that of F_1 's of Mutant Intermediate × *cernuum*. Plant 798 had a mean index of 3.1. An F_2 was grown, and back-crosses to Burma Lacinated and to *cernuum*. Frequency arrays of mean index are given in Table XVIII. In F_2 there were no plants with mean indices higher than 3.2. The back-cross to Burma Lacinated was not examined in the seedling stage. When mature there were plants with lacinated, narrow, and intermediate broad leaves. Some of the plants with mean indices between 3.0 and 3.8 were clearly mosaics. There were 34 plants out of 122, or 28 per cent., with mean indices of 4.3 or higher. This compares with 50 out of 170, or 29 per cent., with mean indices of 4.4 or higher in the back-cross of (Intermediate Mutant × *cernuum*) × Burma Lacinated.

The back-cross to *cernuum* may be divided at 3.0 giving:

Intermediate broad		Narrow		Total
Brown	White	Brown	White	
30	12	10	14	66

Both narrows and whites were deficient, there being 42 broad : 24 narrow, and 40 brown : 24 white. A similar deficiency of white-linted plants occurred in the back-cross of (Mutant Broad × *cernuum*) × *cernuum*. No explanation can be offered. The deficiency of narrows is no doubt due to mutation of **Ll** heterozygotes to homozygous recessive broad. Nine plants of the back-cross were selfed and progenies grown. Frequency arrays are given in Table XVIII. Four types of family may be expected:

(1) **L¹L** heterozygotes, segregating into narrow and intermediate broad.

(2) **L¹l** heterozygotes segregating into broad and intermediate broad.

(3) **Ll** heterozygotes segregating into narrow and broad.

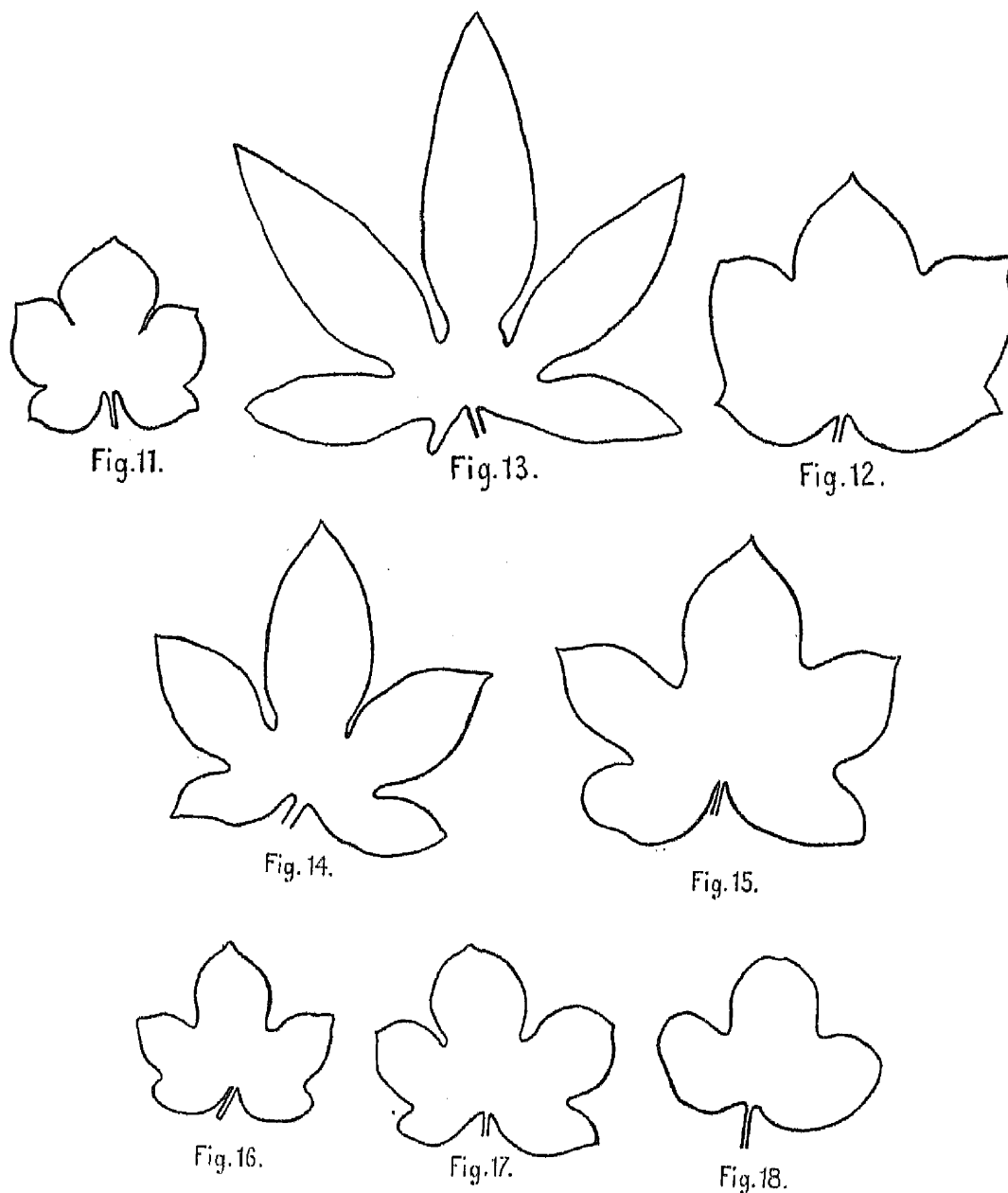
(4) **ll** homozygotes giving broad only.

Type (1) is represented by the progeny of plant 292, which had a mean index of 2.8 and brown lint, and gave four classes of offspring:

	2.2-3.1		3.1-4.2		Total
	Brown	White	Brown	White	
Observed	36	9	4	7	56
Expected (30% c.o.)	34.9	7.1	7.1	6.9	56.0

Evidently, little or no mutation occurred in the **L¹L** heterozygote. Types (2) and (4) are represented by the progenies of plants 279, 283, 284, 295 and 296, which had mean indices of 2.6-2.9. These two types can only be distinguished when they are segregating for brown lint. In **ll** homozygotes (type 4) there will be no association between lint colour and mean index. In **L¹l** heterozygotes, the brown-lint class should have on the whole higher mean indices than the white-lint class, since the **l** gene must

have been derived by mutation from the white-linted *cernuum* parent. Plant 279 was probably an L^1 heterozygote. Type (3) is represented by the progenies of plants 276, 293 and 298, which had mean indices of 3.3



- Fig. 11. Leaf outline of *G. Stocksii*.
 Fig. 12. Leaf outline of F_1 of Mutant Broad \times *G. Stocksii*.
 Fig. 13. Leaf outline of F_1 of Burma Lacinated \times *G. Stocksii*.
 Fig. 14. Leaf outline of F_1 of Cawnpore White \times *G. Stocksii*.
 Fig. 15. Leaf outline of F_1 of Burma Ghost \times *G. Stocksii*.
 Fig. 16. Leaf outline of F_1 of H10 \times *G. Stocksii*.
 Fig. 17. Leaf outline of *G. herbaceum* var. *Wightiana* \times *G. Stocksii*.
 Fig. 18. Leaf outline of *G. herbaceum* var. *africana* \times *G. Stocksii*.

and 3.5. Among the progeny of plant 276 there were no broad-leaved segregates, but since the F_1 plant 798 gave rise to no L gametes, Ll is the only possible constitution. The absence of broad segregates is not surprising in so small a family.

III. THE LEAF SHAPE OF HYBRIDS OF *G. STOCKSII*.

G. Stocksii is a wild species native to the arid regions of Sind and South-east Arabia. It has very small leaves, resembling on a small scale those of *G. herbaceum* var. *Wightiana* (see Fig. 11).

G. Stocksii crosses with cultivated Asiatic cottons fairly readily, but all hybrids investigated have proved completely sterile and some have been vegetatively abnormal. It has only been possible, therefore, to examine a series of F_1 's between *G. Stocksii* and different members of the leaf-shape multiple allelomorph series. A series of leaf outlines for different F_1 's is given in Figs. 12-18. All the F_1 's figured had normal-looking leaves except *Rustenberg* \times *Stocksii*, the leaves of which were of irregular shapes. The influence of *G. Stocksii* is visible in reduced laciniation and leaf lobes with rounded tips and constricted bases in the F_1 's carrying L^L , L and l , whereas in the F_1 of Mutant Broad \times *Stocksii*, carrying L^B , the leaf is somewhat shorter, but the lobe shape shows no effect of the *Stocksii* genotype. Mean index distributions for the F_1 's and for *G. Stocksii* are given in Table XIX.

While it is not possible to demonstrate genic similarity between *G. Stocksii* and cultivated Asiatic cottons, it is clear that the reactions of the leaf-shape genes are of the same order on *G. Stocksii* hybrid genotypes as on cultivated Asiatic genotypes.

IV. THE RATE OF MUTATION.

A large progeny was grown of Burma Lacinated selfed, and a large F_1 of Burma Lacinated \times 1304 to get an estimate of the rate of mutation of the L^L gene.

1689 seedlings of Burma Lacinated were classified for leaf shape, and gave 1683 lacinated : 6 broad leaved. Of the six broad-leaved seedlings four occurred in one family of 422 plants.

664 normal seedlings were grown to maturity and examined for somatic mutations. Mutations were observed on seven plants, and varied in size from a broad middle lobe on an otherwise normal lacinated leaf to a mutant sector in the main stem involving about two-thirds of the plant.

Lint colour was recorded on 652 plants. All plants had brown lint.

3057 seedlings of the F_1 of Burma Lacinated \times 1304 were classified for leaf shape, and gave 3027 lacinated : 30 broad leaved. Of the 30 broad leaved, 26 occurred in three families containing 197 plants. These three families were from three different 1304 plants pollinated by the same Burma Lacinated plant (A8-C-1). A later cross of 1304 φ \times A8-C-1 σ gave 69 lacinated plants only, and from the reciprocal cross

A 8-C-1 ♀ × 1304 ♂, 260 laciniated seedlings were obtained, and no broad-leaved seedlings.

The other four seedling mutants occurred in three different families.

2760 normal plants were grown to maturity and examined for somatic mutations. Six somatic mutations were discovered, varying in size as on Burma Laciniated selfed, from part of a leaf to a sector of a branch, which gave rise to the chimaera shown in Fig. 19.

2422 plants were classified for lint colour. In all cases leaf-shape mutants had brown lint, but among the laciniated-leaved plants, five white-linted mutants occurred.

The occurrence of four broad-leaved mutants in one family of Burma Laciniated selfed, and 26 broad-leaved mutants in three families of 1304 × Burma Laciniated with a common pollen parent indicates that small somatic mutations occurred a few cell generations before gamete formation. From the crossing records it appears likely that the three families which gave 26 mutants were the result of pollinations with a single flower, so that in both cases the somatic mutation was probably too small to be visible. Omitting the seedling mutants ascribed to somatic mutation in the previous generation, there remain to be ascribed to gametic mutation in Burma Laciniated selfed two mutants in 1685 plants, or one in 1685 gametes, and in Burma Laciniated × 1304, four mutants in 3031 plants, or one in 758 gametes. The numbers are, of course, much too small to give any accurate idea of the mutation rate, but it is probably of the order of 1 in 1000.

Since white lint is recessive to brown lint in *G. arboreum*, mutation in a single brown-lint gene would only have a visible effect in a heterozygote. Hence the absence of white-lint mutants in Burma Laciniated selfed. In the F_1 there were five mutants among 2422 plants, or one in 484 gametes. No family contained more than one lint-colour mutant, so that mutation was probably gametic. Whites actually occurred by gametic mutation in a greater proportion of cases than broads, but with such small numbers, and no significance can be attached to the difference.

Somatic mutations were observed on seven out of 664 plants of Burma Laciniated, or one in 95 plants, and on six out of 2760 plants of the F_1 , or one in 460 plants. For purposes of comparison the rate on homozygous Burma Laciniated plants may be taken as seven out of 1328 L^L genes, against six out of 2760 L^L genes in the F_1 . Testing for homogeneity, $\chi^2 = 2.7$, $n = 1$, $P = 0.1$. While the difference is not significant, it suggests that larger numbers might show that the rate of mutation is lower in crosses with 1304.

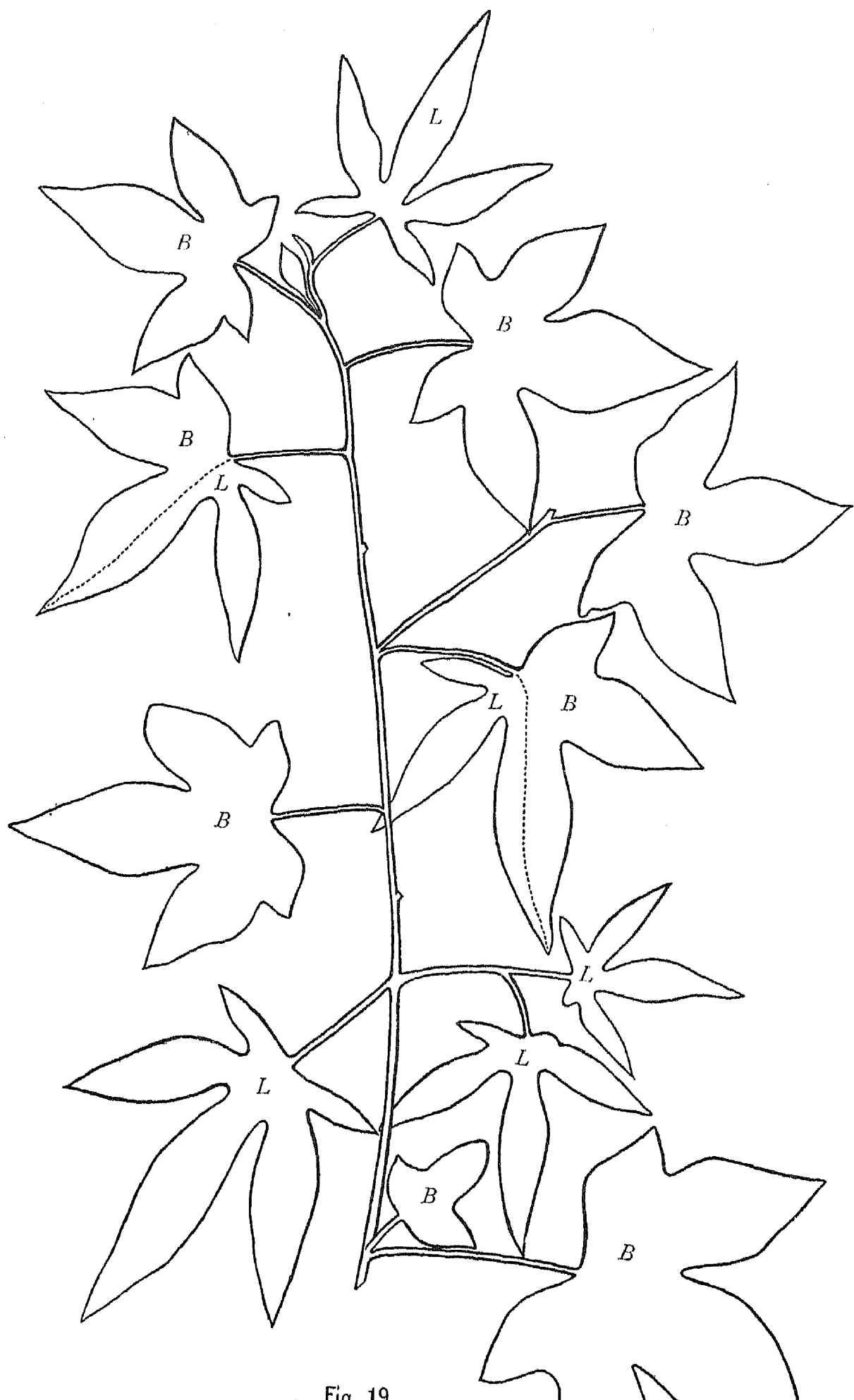


FIG. 19

V. THE RELATION BETWEEN THE LEAF-SHAPE SERIES AND LINT CHARACTERS.

Among the cultivated Asiatic cottons, broad-leaved types have, on the whole, longer and finer lint than narrow-leaved types, and are

TABLE XX.

Lint characters of parents and F₁'s.

Type	Leaf shape constitution	Lint length	Lint index	Seed weight	Lint %
Parents:					
B.L.	LLLL	24	1.4	4.7	29
24-2	LL	29	2.5	6.3	40
C.W.	LL	22	4.3	7.1	61
A 15	LL	26	2.6	6.2	42
<i>cernuum</i>	LL	24	7.3	8.7	85
M.D.	ll	29	4.4	8.4	52
B.G.	ll	27	3.6	6.9	52
1304	ll	29	3.3	7.4	45
N 289	ll	28	2.4	8.3	29
H 9	ll	28	2.2	7.7	29
H 10	ll	27	2.7	7.4	37
F ₁ 's:					
B.L. × G.C. 450	L ¹ L	23	3.2	6.1	53
N 289 × B.L.	LLl	29			
M.D. × G.C.	Li	28	5.1	8.0	64
C.W. × N 289	Li	34	3.8	9.3	41
H 9 × 24-2	Li	32	3.3	7.4	45
H 9 × C.W.	Li	25	3.8	7.8	48
H 10 × C.W.	Li	28	3.5	7.2	49

TABLE XXI.

Mean lint lengths of leaf shape classes.

Cross	Leaf shape allelomorphs	No. of plants examined	Mean narrow	Mean broad	Difference	P
(H 9 × 24-2) × H 10	L × l	23	28.8	30.2	-1.4	0.05
(H 9 × C.W.) × B.G.	L × l	43	25.3	27.0	-1.7	Very small
(H 9 × C.W.) × H 10	L × l	16	27.8	27.3	+0.5	0.6
(H 10 × C.W.) × B.G.	L × l	42	26.0	27.0	-1.0	0.03
(H 10 × C.W.) × H 10	L × l	32	26.9	26.8	+0.1	Large
C.W. × N 289 F ₂	L × l	47	24.0	25.8	-1.8	0.06
N 289 × B.L. F ₂	LL × l	86	26.9	26.7	+0.2	0.4
(N 289 × B.L.) × C.W.	LL × l	21	26.1	27.4	-1.3	0.05
(N 289 × B.L.) × 1304	LL × l	20	28.0	29.4	-1.4	Very small
M.D. × C.W. F ₂	L × l	300	27.2	26.6	+0.6	0.05
M.D. × G.C. 450 F ₁	L × l	59	28.5	28.6	-0.1	0.85
(M.D. × G.C. 450) × M.D.	L × l	32	29.5	29.3	+0.2	0.65
(B.L. × G.C. 450) 801 F ₂	LL × L	35	25.7	26.6	-0.9	0.05
(B.L. × G.C. 450) 801 × G.C. 450	LL × L	71	24.3	24.6	-0.3	0.4
(B.L. × G.C. 450) 801 × A 15	LL × L	57	27.1	27.7	-0.6	0.21
(B.L. × G.C. 450) 801 × 1304	LL × L	26	26.4	27.8	-1.4	0.01

generally supposed to have a lower ginning outturn. Information concerning lint characters was collected as opportunity offered, therefore, during the course of the experiments on leaf shape. Owing to their fragmentary nature, frequency arrays will not be reproduced. The information is summarised in Tables XXI to XXIV as means for two leaf-

shape classes in each cross. In the last two columns are tabulated the differences between the means of the two classes, and *P*, the probability of obtaining a difference as large or larger on random sampling. Where large numbers were available, standard deviations were calculated and *P*

TABLE XXII.

Mean lint indices of leaf shape classes.

Cross	Leaf shape allelomorphs	No. of plants examined	Mean narrow	Mean broad	Difference	<i>P</i>
(H 9 × C.W.) × B.G.	L × l	19	3.19	3.47	-0.28	0.6
(H 9 × C.W.) × H 10	L × l	10	3.10	2.40	+0.70	0.01
(H 10 × C.W.) × B.G.	L × l	29	3.58	3.46	+0.12	Large
(H 10 × C.W.) × H 10	L × l	11	3.42	3.46	-0.04	Large
M.D. × G.C. 450 <i>F</i> ₂	L × l	45	5.45	5.23	+0.22	0.5
(M.D. × G.C. 450) × M.D.	L × l	25	4.98	5.37	-0.39	Very small
(B.L. × G.C. 450) 801 × G.C. 450	LL × L	20	3.95	4.36	-0.41	0.3
(B.L. × G.C. 450) 801 × A 15	LL × L	22	3.28	3.40	-0.12	0.7

TABLE XXIII.

Mean seed weights for leaf shape classes.

Cross	Leaf shape allelomorphs	No. of plants examined	Mean narrow	Mean broad	Difference	<i>P</i>
(H 9 × C.W.) × B.G.	L × l	19	6.28	7.54	-1.26	Very small
(H 9 × C.W.) × H 10	L × l	10	7.45	6.45	+1.00	Very small
(H 10 × C.W.) × B.G.	L × l	29	6.33	7.04	-0.71	0.01
(H 10 × C.W.) × H 10	L × l	11	7.18	7.10	+0.08	0.8
M.D. × G.C. 450 <i>F</i> ₂	L × l	45	7.43	7.10	+0.33	0.35
(M.D. × G.C. 450) × M.D.	L × l	25	7.75	8.41	-0.66	Very small
(B.L. × G.C. 450) 801 × G.C. 450	LL × L	20	5.63	5.40	+0.23	0.6
(B.L. × G.C. 450) 801 × A 15	LL × L	22	5.88	6.08	-0.20	0.45

TABLE XXIV.

Mean of lint percentage for leaf shape classes.

Cross	Leaf shape allelomorphs	No. of plants examined	Mean narrow	Mean broad	Difference	<i>P</i>
(H 9 × C.W.) × B.G.	L × l	19	51.1	46.7	+4.4	0.25
(H 9 × C.W.) × H 10	L × l	10	41.8	37.2	+4.6	0.2
(H 10 × C.W.) × B.G.	L × l	29	56.7	49.5	+7.2	0.01
(H 10 × C.W.) × H 10	L × l	11	51.5	47.6	+3.9	0.6
M.D. × G.C. 450 <i>F</i> ₂	L × l	45	73.9	73.7	+0.2	Large
(M.D. × G.C. 450) × M.D.	L × l	25	64.2	63.8	+0.4	0.85
(B.L. × G.C. 450) 801 × G.C. 450	LL × L	21	71.3	80.1	-8.8	0.1
(B.L. × G.C. 450) 801 × A 15	LL × L	22	55.2	56.4	-1.2	0.8

determined from the table of the deviation in the normal distribution in terms of the standard deviation given by Fisher (1932, Table I). Where the number of plants in a family was small, *P* was determined by the “*t*” method described by Fisher for the comparison of the means of two small samples (Fisher, 1932). Mean lint characters of parents and *F*₁’s are given in Table XX.

Lint length, Table XXI.

Information is available from eight crosses.

Of three inter-*arboreum* crosses, narrow-leaved plants had slightly, but significantly, longer lint than broad-leaved plants in Million Dollar \times Cawnpore White. In Million Dollar \times *cernuum* 450 there was no difference in lint length between the narrow and broad-leaved classes. In Burma Laciniated \times *cernuum* 450 narrow-leaved plants exceeded laciniated-leaved plants in lint length in F_2 and in back-crosses to *cernuum* 450, A15 and 1304. The differences may therefore be judged to be real.

In seven crosses of *G. arboreum* \times *G. herbaceum*, the broad-leaved classes had significantly longer lint than the narrow-leaved classes in all back-crosses to *arboreum*, in the F_2 of N289 \times Cawnpore White, and in the back-cross of (H9 \times 24-2) \times H10.

In the back-cross of (Cawnpore White \times N289) \times Cawnpore White there was a correlation of $r = -0.125$ (P very small) between lint length and mean index.

Non-significant differences were obtained in the F_2 of N289 \times Burma Laciniated and the back-crosses of (H9 \times Cawnpore White) \times H10 and (H10 \times Cawnpore White) \times H10. *G. herbaceum*, therefore, carries a gene for long lint in the same chromosome as the leaf-shape genes, which is not carried by the *G. arboreum* types tested. In certain back-crosses to *G. herbaceum*, the effect of this gene may be hidden either by dominance of the *herbaceum* gene, or by the effect of other genes. There are probably several allelomorphs of this lint-length gene, since Cawnpore White carries a higher member than Million Dollar, which carries the same as *cernuum*, which, in turn carries a higher member than Burma Laciniated. N289, on the other hand, carries a member of the series which is higher than either Cawnpore White or Burma Laciniated.

Lint index, Table XXII.

The evidence for the existence of genes in the leaf-shape chromosome affecting lint index is inconclusive.

Significant differences in lint index between leaf-shape classes only occurred in back-crosses of (H9 \times Cawnpore White) \times H10, where the narrow class exceeded the broad class, and (Million Dollar \times *cernuum*) \times *cernuum*, where the broad class exceeded the narrow class. In the back-cross of (Cawnpore White \times N289) \times Cawnpore White there was no correlation between lint index and mean index.

Seed weight, Table XXIII.

There was no difference in seed weight between the leaf-shape classes in Burma Laciniated \times *cernuum*. In Million Dollar \times *cernuum* the back-cross showed a significantly greater seed weight in the broad-leaved class, but the difference in the F_2 was in favour of the narrow-leaved class, and non-significant.

In the two crosses H9 \times Cawnpore White and H10 \times Cawnpore White, broad-leaved plants had considerably heavier seeds than narrow-leaved plants in back-crosses to *arboreum*, and in the back-cross of (N289 \times Cawnpore White) \times Cawnpore White, there was a correlation of $r = -0.207$ (P very small) between seed weight and mean index. In the back-cross of (H9 \times Cawnpore White) \times H10 however, narrow-leaved plants had heavier seeds than broad-leaved plants, and in the back-cross of (H10 \times Cawnpore White) \times H10 there was no difference between classes. These results are similar to those obtained for lint length, and suggest that the same gene for long lint carried by *herbaceum* also increases the weight of the seed, at least when transferred to a genotype carrying a large proportion of *arboreum* genes.

Lint percentage, Table XXIV.

Differences in lint percentage between leaf-shape classes were non-significant except in the back-cross of (H10 \times Cawnpore White) \times Burma Ghost. In all four back-crosses of H9 \times Cawnpore White and H10 \times Cawnpore White, however, narrow-leaved plants had about 5 per cent. higher lint percentage than broad-leaved plants, and in the back-cross of (N289 \times Cawnpore White) \times Cawnpore White there was a correlation of $r = +0.282$ between mean index and lint percentage.

Herbaceums, therefore, probably carry a gene for low lint percentage—probably low density of hairs on the seed surface—in the same chromosome as the leaf-shape gene.

VI. LINKAGE RELATIONS.

The linkage between the leaf-shape multiple allelomorph series and the gene controlling brown lint in the *arboreum* species has already been referred to. Data from families in which there was no disturbance of the leaf-shape segregation are summarised below.

Cross	Constitution	AB	Ab	aB	ab	Total	Crossing-over %
Back-crosses							
(B.L. × C.W.) × C.W.	L ^L KLk × LkLk	42	14	16	40	112	26.8
(B.L. × G.C.) 801 × A 15		20	8	10	21	59	30.6
(B.L. × G.C.) 801 × G.C.		30	8	9	25	72	23.6
(B.L. × G.C.) 801 × 1304	L ^L KLk × lkLk	4	4	6	8	22	45.4
(B.L. × 1304) × 1304	L ^L Klk × lkLk	39	22	16	32	109	34.8
(A9 × C.W.) × C.W.	L ^B KLk × LkLk	21	5	10	20	56	26.8
(A9 × A13) × C.W.		70	26	27	72	195	26.2
(A9 × 1304) × C.W.	L ^B Klk × LkLk	77	41	34	74	226	33.2
(A20 × C.W.) 3 × C.W.	L ^L KLk × LkLk	26	12	13	27	78	32.1
(A20 × C.W.) 10 × C.W.	L ^L Klk × LkLk	7	4	5	6	22	40.8
(764 × B.L.) 35 × A24	L ^L Klk × lkLk	82	31	36	94	243	27.6
Total		418	175	182	419	1194	29.9
Expected (29.9 % crossing-over)		418.5	178.5	178.5	418.5	1194.0	
<i>F</i> ₂ 's							
B.L. × G.C. 801	L ^L Klk	17	6	5	8	36	
B.L. × 1304	L ^L Klk	15	1	1	5	22	
A20 × C.W. 3	L ^L KLk	40	11	8	7	66	
764 × B.L.-35	L ^L Klk	39	9	10	6	64	
Total		111	27	24	26	188	
Expected (29.9 % crossing-over)		117.0	23.9	23.9	23.2	188.0	

The results agree well together, and from the totals of the back-cross results the cross-over rate is 29.9 per cent. This fits very well the totals of the *F*₂ results.

The relation of leaf shape with corolla colour was investigated in the *F*₂ of Million Dollar × Cawnpore White. Corolla colour and leaf shape were inherited independently. Results are given below.

	Narrow		Broad		Total
	Yellow	White	Yellow	White	
Observed	717	249	236	80	1282
Expected	720	240	240	80	1280

The relation between the leaf-shape multiple allelomorph series and the anthocyanin multiple allelomorph series was studied in four crosses: Sanguinea × Abu Hareira, Sanguinea × Burma Ghost, Burma Laciniated × 1304, and 31-5 × 1304. Results are summarised below.

Cross		<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>	Total	$\chi^2(L)$	<i>P</i>
Back-crosses								
(G.S. 2 × B.S.) × 1304	RLrg1 × rg1rg1	8	11	12	13	44	0.91	0.3
(G.S. 2 × A.H. 1-9)	RLRs1 × RslRs1	71	49	51	79	250	10.0	very small
× A.H. 1-9								
(B.L. × 1304) × 1304	RsLLrg1 × rg1rg1	22	16	12	11	61	0.41	0.5
31-5 × 1304	RsLLrgL × rg1rg	176	68	68	82	294	1.65	0.2
<i>F</i> ₂ 's								
G.S. 2 × B.S.	RLrg1	136	24	41	10	211	0.51	0.5
G.S. 2 × A.H. 1-9	RLRs1	106	27	33	14	180	1.67	0.2
B.L. × 1304	RsLLrg1	9	4	4	1	18	0.22	0.6

Results from the back-cross (G.S. 2 \times Abu Hareira 1-9) \times Abu Hareira 1-9 alone indicate linkage with 40 per cent. crossing-over. With 40 per cent. crossing-over the F_2 ratio would be:

$$106 \text{ RL} : 29 \text{ Rl} : 29 \text{ R}^s \text{ L} : 16 \text{ R}^s \text{ l},$$

a close approach to the observed ratio. The observed deviations from free assortment in the F_2 are, however, not significant. In the two crosses G.S. 2 \times B.S. and Burma Laciniated \times 1304 there was no evidence of linkage. In 31-5 \times 1304 there was a slight but non-significant excess of non cross-over classes.

If the **R** series and the **L** series are situated in the same chromosome, they are so far apart as to assort freely in most cases.

Since **K** is in the same chromosome as **L**, the relation between **K** and **R**^s in Burma Laciniated \times 1304 is given below.

	RsK	Rsk	rgK	rgk	Total	$\chi^2 (L)$	<i>P</i>
F_2 (normal and mutant)	25	10	15	0	50	5.56	0.02
$F_1 \times 1304$ (normal and mutant)	19	26	19	16	80	1.25	0.25

In both F_2 and back-cross there is an excess of cross-over classes. Numbers are too small for any importance to be attached to them.

SUMMARY OF EXPERIMENTAL RESULTS.

1. The main differences in leaf shape in Asiatic cottons result from the action of a multiple allelomorph series of five numbers, **L**^B, **L**^I, **L**^L, **L**, and **l**.

2. Of these, **L**^B and **l** give dominant and recessive broad respectively. **L**^I gives dominant intermediate broad. **L**^L gives laciniated, and **L** narrow leaf.

3. **L**^L, **L** and **l** give intermediate heterozygotes. **L**^B and **L**^I are dominant over all other members of the series.

4. **L**^L, **L** and **l** occur in nature in *G. arboreum* and its varieties. **L**^B and **L**^I arose by mutation in cultures of a laciniated *arboreum* strain. All *G. herbaceum* varieties so far reported carry **l**.

5. The differences in leaf shape of taxonomic value are differences in minor genes affecting such characters as lobe shape, leaf size, and rumpling, and not laciniation.

6. The leaf-shape multiple allelomorph series is linked with a gene for brown lint (**K**), with about 30 per cent. crossing-over.

7. The Burma Laciniated strain is mutable at the **L** locus and at the **K** locus. **L**^L mutated to **L**^B, **L**^I and **l**. Mutation occurred in about 1 per 1000 of **L**^L gametes, in about 1 per cent. of homozygous **L**^L plants, and in

one in 450 plants of Burma Lacinated \times 1304 F_1 . **K** mutated to **k** in about one in 500 gametes. **L**^I mutated to **L**^B, and **L**^B mutated to **l**. In one case mutation from **L**^B to **l** was accompanied by mutation from **K** to **k**.

8. *Cernuum* is mutable in the **L** gene. Mutation was observed from **L** to **l**, and evidence is presented to show that mutation probably occurred from **L**^L to **L**. No dominant mutants were obtained from *cernuum*.

9. Mutation occurred from **L** to **l** in two *arboreum* types, Cawnpore White and A13, in heterozygotes with Mutant Broad.

10. No mutation has been discovered in recessive broad.

11. Mosaics and chimaeras of two different leaf-shape genotypes were observed in several crosses. Mosaics were very unstable, and changed rapidly by further mutation to homogeneous mutant types.

12. F_1 hybrids of *G. Stocksii* by four of the five leaf-shape allelomorphs had leaf indices of the same order as in cultivated Asiatic types.

13. Linkage exists between the leaf-shape allelomorph series and genes affecting lint length, seed weight and lint percentage.

14. The **L** series and the corolla colour (**Y**) series assort freely. There appeared to be linkage between the **L** series and the anthocyanin (**R**) series in a single cross. In other crosses the two allelomorph series assorted freely.

DISCUSSION.

The existence of a single main factor responsible for the difference in leaf shape between the narrow-leaved arboreums and the broad-leaved arboreums and the herbaceums has been established.

On the basis of the difference in leaf shape previous workers have separated broad-leaved forms from *G. arboreum* under the name of *G. Nanking* or *G. indicum*. The genetic evidence shows that the difference is of no greater importance than differences in corolla colour (see Hutchinson, 1931) or anthocyanin pigmentation (see Hutchinson, 1932*b*), and should therefore be regarded as of no taxonomic value. Such segregation as occurs in minor factors is of little importance, and the whole of the difference in leaf shape can be expressed in terms of depth of lacination.

In crosses of *G. arboreum* \times *G. herbaceum*, on the other hand, the effect of segregation of minor factors is of greater importance. The dividing line between narrow and broad classes is less distinct, and the frequency arrays of mean index are less regular. In Leake's (1911) cross of *G. herbaceum* var. *Wightiana* \times *G. arboreum* the dividing line was entirely obscured. It is not possible to express the whole of the difference in leaf shape between *arboreum* and *herbaceum* in terms of lacination.

Rounded versus pointed lobes and the presence or absence of constriction at the base of the lobe, are characters which affect the leaf shape but which are not due to differences in laciniation. The factors controlling these characters and the laciniation modifier group segregate in crosses of *Nanking* \times *herbaceum*, giving rise to arrays in F_2 which transgress the parental distributions. This is best shown in the crosses between the herbaceums H9 and H10 and *arboresum* types, where the F_1 's were back-crossed both to H10 and to Burma Ghost. The back-cross parents were both recessive broad (ll) types, but the difference in the modifier complex caused a very considerable shift in the frequency arrays of narrow and broad segregates alike.

These minor leaf-shape factors can be used in systematic work, since their distribution is the same as that of other groups of factors which distinguish the genotypes of *G. arboresum* and *G. herbaceum*.

The distribution of the allelomorphic series is of interest. In *G. herbaceum* and its varieties one allelomorph only—recessive broad—has been found, and variations in leaf shape within the species are confined to variations in lobe shape which have little or no effect on laciniation. In *G. arboresum*, on the other hand, the allelomorphs L and l are about equally common. Lacinated L^L occurs in Assam and Burma, but is apparently not common except in *cernuum*. The dominant mutant allelomorphs have not been recorded previously, but since they would not be distinguished from recessive broad in the field, it is quite possible that they may occur.

The leaf shape of hybrids of *G. Stocksii* suggests that the *G. Stocksii* gene complex is more similar to *G. herbaceum* than to *G. arboresum*, since it has the effect of broadening all types except dominant broad.

The information obtained on the relation between lint characters and leaf shape suggests that a fraction of the lint length, seed weight, and lint percentage variances are due to genes situated in the leaf shape-brown lint chromosome. This is in agreement with previous results (Hutchinson, 1931 and 1932b) in showing that lint characters are influenced by a considerable number of genes of small individual effect scattered through the chromosome complement.

The behaviour of the multiple allelomorphic series throws considerable light on the organisation of the constituent genes. The series is made up of two rather distinct groups, (1) L^L , L and l, the laciniation series, having intermediate heterozygotes, and (2) L^B and L^I , two genes responsible for more or less broad leaves, which are almost completely dominant over all other members of the series.

(1) *The laciniation series.*

The best estimates of the mean value of the mean index for a comparable series of genotypes involving L^L , L and l are provided by the back-crosses of Burma Laciniated \times N289 to Cawnpore White and to 1304 (Table VI). In these two back-crosses mean index distributions are given for the genotypes $L^L L$, $L^L l$, Ll and ll , while the F_1 Burma Laciniated \times N289 provides a further estimate of $L^L l$. The following are the means and standard deviations of the four genotypes:

Cross	Genotype	Mean of mean index	$\sigma (M)$
(B.L. \times N289) \times C.W.	$L^L L$	6.59	0.190
(B.L. \times N289) \times 1304	$L^L l$	6.11	0.267
B.L. \times N289 F_1	$L^L l$	6.00	—
(B.L. \times N289) \times C.W.	Ll	3.21	0.078
(B.L. \times N289) \times 1304	ll	2.58	0.038

To these may be added statistics for Cawnpore White and Burma Laciniated. These, though not so closely comparable as the above series, provide estimates of $L^L L^L$ and LL .

Type	Genotype	Mean of mean index	$\sigma (M)$
Burma Laciniated	$L^L L^L$	8.38	0.312
Cawnpore White	LL	4.06	0.095

The mean of the $L^L L^L$ group is depressed by two unusually low values, 6.9 and 7.0.

The difference between LL and ll is 1.48, and between $L^L L^L$ and ll is 5.48, almost exactly four times as great. The difference between Ll and ll is 0.63, or very nearly half the difference between LL and ll . Similarly, the F_1 of Burma Laciniated \times N289 and the mean of the $L^L l$ group from (Burma Laciniated \times N289) \times 1304 are near the mean of $L^L L^L + ll$ homozygotes, and the $L^L L$ group from (Burma Laciniated \times N289) \times Cawnpore White is near the mean of $L^L L^L + LL$ homozygotes, and is higher than ll by about five-eighths of the difference between $L^L L^L$ and ll .

These relationships are represented graphically in Fig. 20, where the mean values of mean index for the six genotypes are plotted against ordinates calculated on the assumptions that (1) an L gene differs from an l gene in one unit, and an L^L gene differs from an l gene in four units; and (2) that genes in homologous chromosomes are additive in their effects. The genotypes will then differ from the basal ll genotype as follows:

Genotype	Number of Units
ll	0
Ll	1
LL	2
$L^L l$	4
$L^L L$	5
$L^L L^L$	8

The seven values fall very nearly on a straight line.

The regression line calculated from the data by the formula

$$Y = a + b(x - \bar{x}),$$

where $a = \bar{y} = 5.15$ and

$$b = \frac{s\{y(x - \bar{x})\}}{s\{(x - \bar{x})^2\}} = 0.74.$$

is plotted on the same figure for comparison.

To these units by which the genes differ, Thompson's (1931) term "episomes" may be applied.

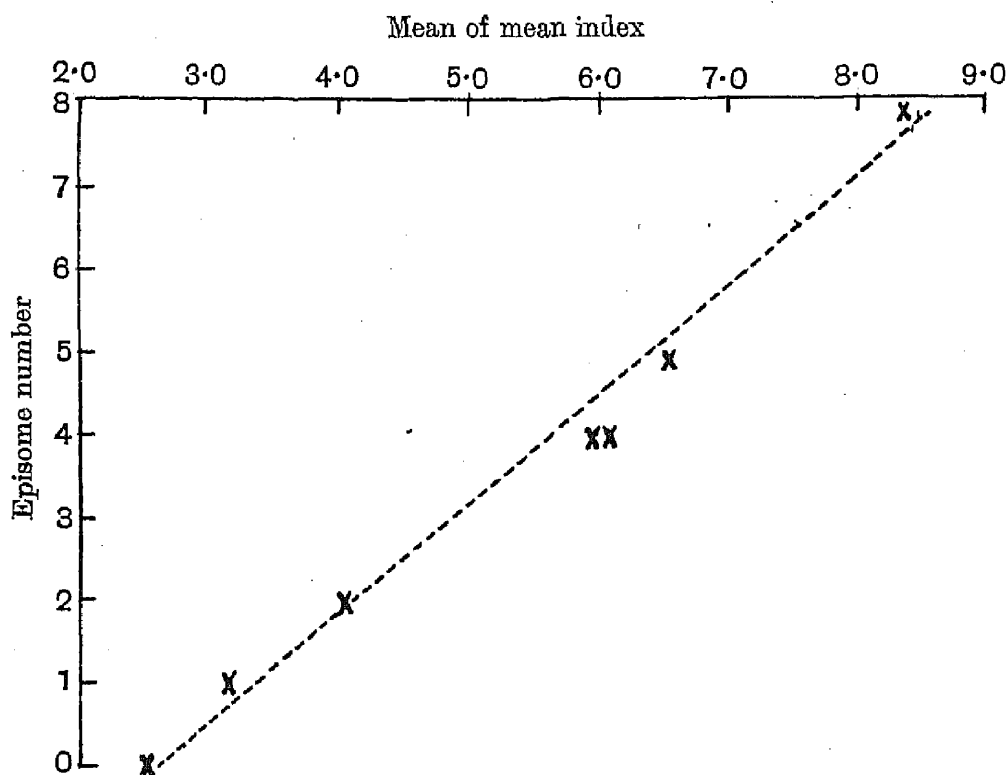
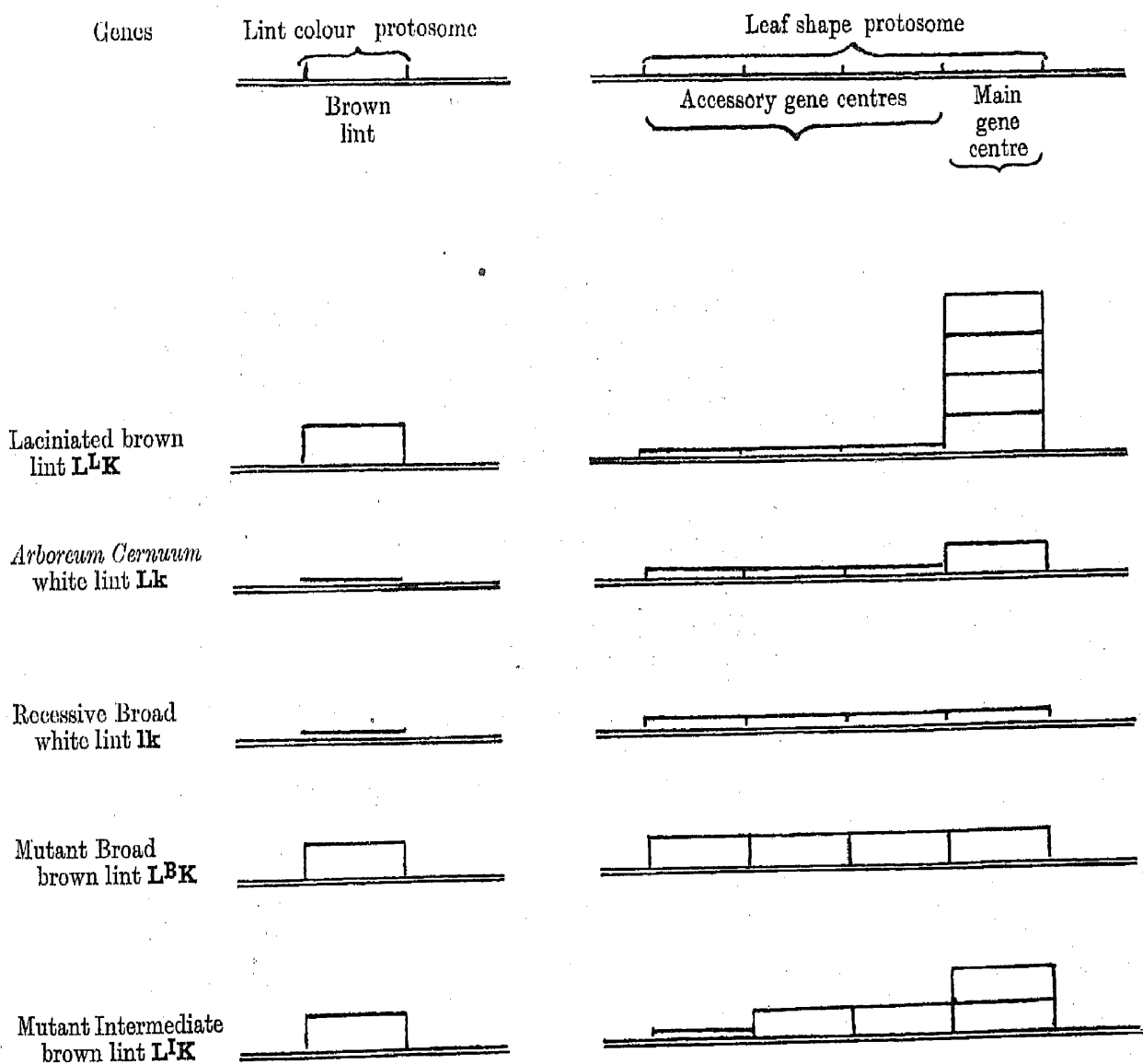


Fig. 20. Relation between mean of mean index distributions and episome number. Back-crosses of Burma Laciniated \times N 289. $Y = 5.15 + 0.74(x - 3.33)$.

An understanding of the relationship of the dominant genes to the laciniation series depends on an understanding of the significance of dominance. Fisher (1928*a*, 1928*b*, 1929) and Harland (1932*b*) have shown that dominance is, in some cases at least, the result of the action of modifying genes, and not of the gene itself. In this case, since dominant broad is an allelomorph of recessive broad, dominance is undoubtedly a function of the gene. It may be supposed, therefore, that mutation from L^L to L^B involves a rearrangement of the L^L gene which gives it an effect on leaf shape similar to that of the l gene, and at the same time reinforces that effect in a manner similar to that of independent genes modifying dominance. In addition, since the direction of mutation is at least

usually, and possibly invariably, from narrower to broader, any hypothesis of the organisation of the gene should ascribe a more stable arrangement to broad genes than to narrow genes. All these requirements are fulfilled by supposing that the change from L^L to L^B involves a rearrangement of the four units, or episomes, from a chain arrangement to a side by side arrangement in the gene base, or "protosome" to use Thompson's (1931) term. This hypothesis involves Agol's (1931) and Dubinin's (1932) theory that the protosome is subdivided into a number of adjacent "gene centres" arranged in linear order along the axis of the chromosome, and it will be argued below that the scheme here presented is capable of unifying the theory of "step allelomorphism" with the "side chain" theory developed by Thompson.

Diagrams of the suggested organisation of the leaf shape genes are given below:



Then, in crosses involving the lacination series, the shape of the leaf is the result of the additive effect of the episomes on the main gene centre, and the rest of the protosome is inactive. L^B gives a broad leaf because the episome level is the same on the main gene centre as on the accessory gene centres, and L^I gives a somewhat narrower leaf because the episome level is higher on the main gene centre than on the accessory centres. The accessory gene centres, which are active in these two allelomorphs, act in a manner similar to independent modifying genes in reinforcing the effect of the heterozygote, and thereby inducing dominance of the mutant genes.

The mutations observed fit the theory of the gene organisation satisfactorily. Mutant broad and mutant intermediate arise by simple rearrangement from lacinated, and recessive broad by simple loss of the whole episome chain from lacinated. Further rearrangement in mutant intermediate gives mutant broad and loss of episomes from mutant broad gives recessive broad. Mutation in lacinated involves redistribution of a relatively long chain on a single centre, and occurs independently of mutations involving loss of the episome on the lint-colour gene centre. The mutation observed in mutant broad, involved loss of single episomes from a series of gene centres, and from the brown-lint protosome at the same time. That this was not a case of deficiency is shown by the facts that no sterility resulted even in homozygotes, and that crossing-over between leaf shape and brown lint remained normal. The more widely spread the effect in a single gene, the more likely it will be that other genes will be affected, and coincident mutations in leaf shape and lint colour would therefore be more likely to occur in an $L^B K$ than an $L^I K$ chromosome. Mutation in L genes should give l only, as, in fact, they did.

Mutation from recessive broad to another member of the series could only be expected if there were some mechanism for the acquisition of episomes. Reverse mutation from a dominant mutant to lacinated would involve the piling up of episomes on a single centre, and may be expected to be a much less common occurrence than the redistribution of a chain over all centres.

It has not yet been possible to accumulate much evidence on the causes of the instability of *cernuum* and Burma Lacinated. Some information is given by a comparison of the behaviour of two types of $L l$ heterozygotes. In the cross of Burma Lacinated \times 1304 no mutants were observed in F_2 or in a back-cross to 1304 (see Table VI). In the F_1 somatic mutations occurred on six plants out of 2760. No mutants were observed in back-crosses to Cawnpore White and 1304 of Burma Lac-

initiated \times N289. In F_2 two plants with mean indices of 4.2 in a family of 156 are the only ones which may possibly have been mutants. Four progenies were grown from selfed seed of $L^L I$ heterozygotes from the back-cross of 764 \times Burma Laciniated, and one progeny from a back-cross of (764 \times Burma Laciniated) 35 by a recessive broad extracted from it. These five progenies had nothing but Burma Laciniated and its mutants in their ancestry. In the four progenies from selfed seed there were 22 plants out of 214 with mean indices between 2.8 and 4.1, which were no doubt mutants, and one mutant plant was observed in a family of 243 from 764 \times Burma Laciniated — 35 \times extracted recessive broad. Of the 22 mutants in the selfed progenies, 17 occurred in a family of 101 plants from 764 \times Burma Laciniated — 39 (see Table VIII). The rate of somatic mutation is greater in homozygous Burma Laciniated than in the F_1 of Burma Laciniated \times 1304, though not significantly so. These facts agree in suggesting that the mutation rate depends to a considerable extent on genes independent of the main leaf-shape gene. The results from the progenies of 764 also suggest that Burma Laciniated may itself be heterozygous for genes inducing mutation.

The direction of mutation, on the other hand, depends on the allelomorphs present. Homozygous $L^L L^L$ mutates to one or other of the dominant genes, L^B or L^I . $L^L L^B$ mutates to $L^B L^B$ in all cases. $L^L L^I$ mutates to $L^I L^I$ in all, or nearly all cases. $L^L I$, however, mutates to $L^B I$, $L^I I$, or II .

L , whether from *arboreum* or from *cernuum*, is only known to mutate to l .

A hypothesis is useful in so far as it unifies a body of observed fact under a single causative mechanism, and in so far as it can be used for and tested by the successful prediction of further facts.

The published work on multiple allelomorphic series and mutable genes can all be unified on the present theory of gene architecture, and certain predictions can be made from it which are amenable to experimental verification.

(2) *Pericarp and cob colour in maize.*

Emerson (1914) made the first studies on variegated pericarp in maize. He studied mutation from variegated to red, and found that the greater the amount of red, the greater the chance that the zygote carried red. In a later paper (1929) he showed that heterozygous variegated (VW) had a higher mutation rate than homozygous variegated (VV). He found that the rate of mutation was affected by the white parent, and suggested that

modifiers of mutation rate existed in the same chromosome as the pericarp allelomorph series.

Pericarp colour in maize is controlled by a multiple allelomorph series, a complete list of which is given by Anderson (1924) and reproduced below:

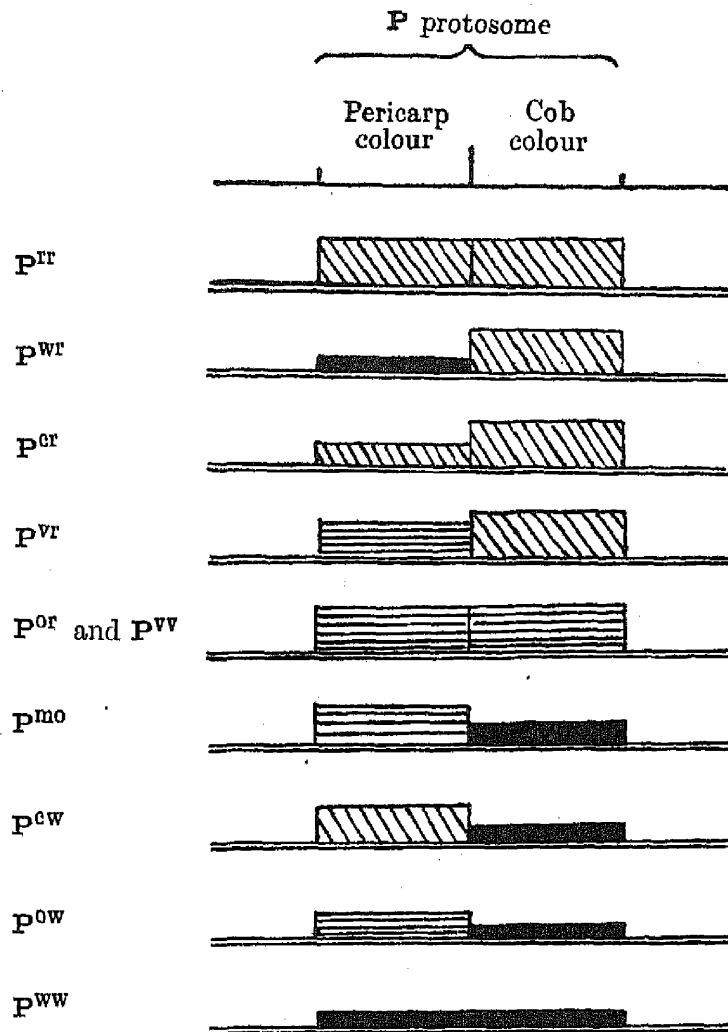
Gene	Pericarp colour	Cob colour
Prr	Red	Red
Por	Light red or orange	Light red or orange
Pwr	White or pale orange	Red
Pow	Light orange	White
Pcw	Red or orange at base, with white cap	White
Pcr	" "	Red
Pww	White	White
Pvv	Variegated	Variegated
Pmo	Mosaic	Light or white
Pvr	Variegated	Red

Eyster (1924, 1925) studied the inheritance of orange pericarp (**P^{ow}**) and mosaic pericarp (**P^{mo}**). He concluded that they formed two groups of mutable allelomorphs in the pericarp colour multiple allelomorph series, each group being made up of several members controlling different intensities of colour and pattern. Mutation within the allelomorph group was very frequent. Mosaic also mutated to orange, red and colourless, and orange to mosaic, red and colourless. Red mutated with low frequency to mosaic and orange. White was invariably stable. Orange appeared to be dilute red uniformly distributed, and mosaic a similar amount of red distributed in patches. If orange mutated to mosaic, the intensity of the mosaic was of the same grade as that of the parent orange. Eyster suggested that the gene consists of gene elements, some pigment producing, and some not, the intensity of colour being dependent on the relative proportions of the two. The theory does not account for the fact that red mutates occasionally whereas white is invariably stable. Since red and white should each contain only one kind of gene element, both should be stable.

Anderson and Eyster (1928) studied the rate of somatic mutation of variegated pericarp to red, and found that the rate increased greatly in the last cell generations of the development of the seed.

Eyster's theory may be simplified and brought into line with the present theory by postulating one kind of "gene element" or episome—colour-producing—only. Then red has a full complement. White has none, and is therefore of necessity stable. To account for the large number of allelomorphs of orange and mosaic it is necessary to postulate a large number of small episomes, and to suppose that a complete set—the red allelomorph—is arranged in a fairly stable manner, while incomplete

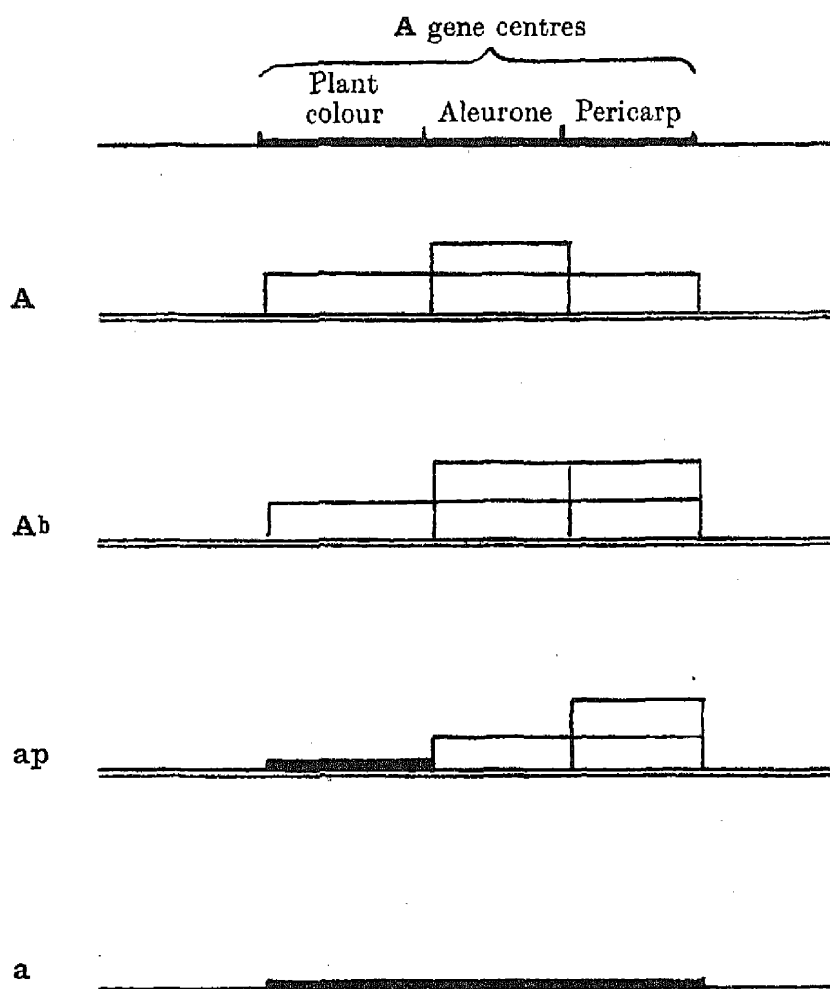
sets are unstable and give orange or mosaic according to the type of arrangement. Changes in the number of episomes cause changes in the intensity of orange or mosaic, while the completion of a set gives fairly stable red, and complete loss of a set gives stable white. While there is no crossing-over between cob colour and pericarp colour, reference to the list of **P** genes given above shows that they are independent of one another



in their effects. Red cob may be associated with red, white, red with white cap or variegated pericarp, and white cob may be associated with orange, red with white cap, mosaic or white pericarp. The **P** series of allelomorphs behaves in fact as though it were made up of two separate genes lying side by side in the chromosome. Since both pericarp and cob allelomorphs have the same effects—red, orange, variegated and white are common to both—there is clearly more in common between them than an accident of position. On the present theory they are two gene centres of the same protosome. Evidence that the episomes attached to the two gene centres are identical is provided by Eyster (1924), who records that

orange pericarp white cob mutated to variegated pericarp, variegated cob. Since there is no evidence that episomes can be built up *de novo* on a gene centre with none, it must be supposed that the change from orange to variegated on the pericarp colour gene centre was accompanied by a redistribution of episomes by which some were transferred to the neighbouring cob colour gene centre.

Diagrams of the suggested organisation of the **P** allelomorphs are given on page 501.

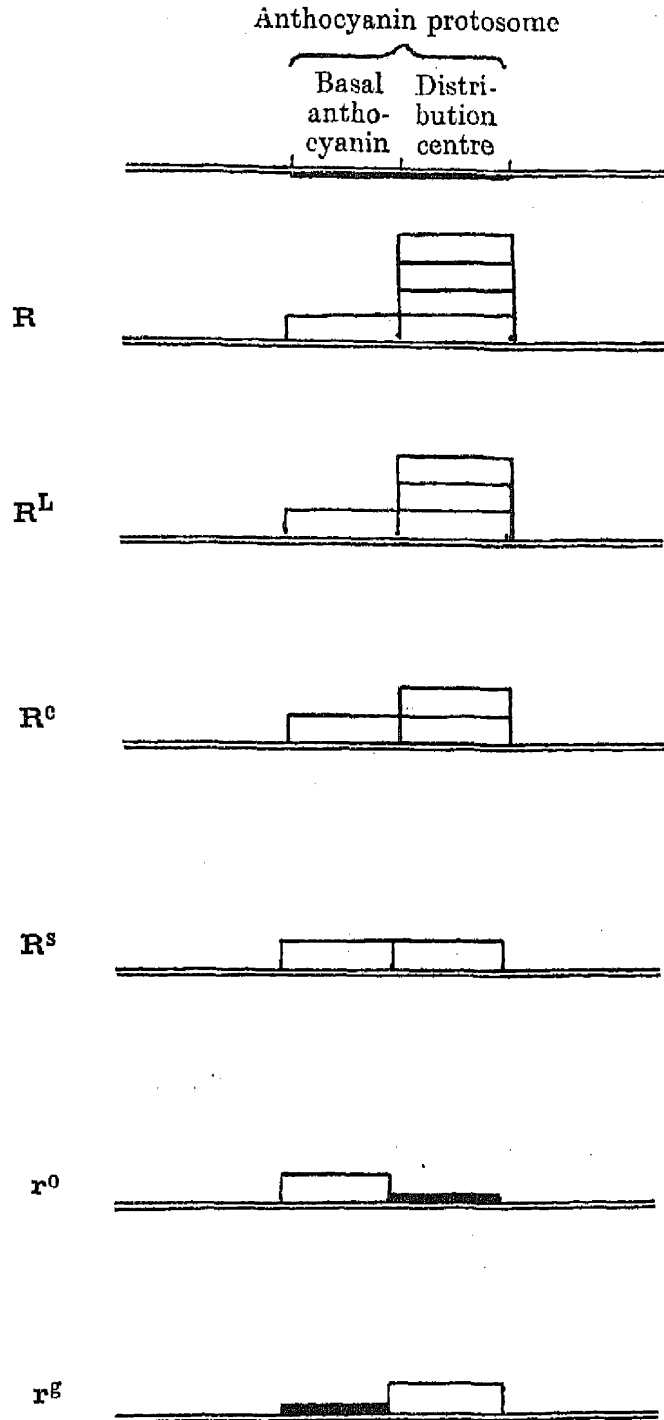


Diagonal hatching indicates a stable arrangement, and horizontal hatching an unstable arrangement of episomes. **P^{ew}** and **P^{cr}** are reported to be stable, and they have accordingly been figured with a stable intermediate episome block. The orange and variegated groups of allelomorphs are represented by a single diagram. The range of allelomorphs responsible for different intensities of orange and variegated would be represented by a series of larger and smaller episome blocks.

(3) *The A basal colour series in maize.*

Emerson and Anderson (1932) describe a series of four allelomorphic genes for colour in maize, the effects of which can be interpreted on the

present theory of gene organisation. Three gene centres are involved, plant colour, aleurone colour, and pericarp colour. The basal colour gene **A** has two episomes attached to the aleurone gene centre and one to each of the other gene centres. The brown pericarp allelomorph **A^b** has a single



episome attached to the plant colour gene centre, and two episomes attached to each of the other gene centres. The allelomorph **a^b** which is almost like recessive **a** in its effect on plant colour, and gives a pale aleurone colour with **C** and **R**, but which gives dominant brown colour in the pericarp, has no episome attached to the plant colour gene centre, one

to the aleurone centre, and two to the pericarp centre. White, *a*, has no episomes. Diagrams of the suggested organisation of the **A** genes are given on page 502.

(4) *The anthocyanin multiple allelomorph series in cotton.*

The anthocyanin multiple allelomorph series in Asiatic cottons described by the present author (Hutchinson, 1932*b*) can be represented on this scheme by two gene centres with a series of episomes attached (see p. 503).

The complementary nature of the r^0 and r^s allelomorphs is satisfactorily accounted for, since the r^0 and r^s heterozygote carries the basal anthocyanin episome and the spot episome, and would therefore be expected to be **R^s**. The step by step restriction of anthocyanin to fewer and fewer parts of the plant is represented as the effect of progressive reduction in the number of episomes on a single gene centre.

A further series of genes in the **R** series may be predicted as theoretically possible on the above scheme of organisation. By loss of the basal anthocyanin episome from **R**, **R^L**, and **R^c**, three new genes would be formed which should, when homozygous, all give Ghost Spot types, and in heterozygotes with r^0 should give red, red leaf, and red calyx heterozygotes respectively. A further theoretical possibility is a gene without episomes, which would presumably give a Spotless homozygote without any anthocyanin, and would give with r^0 a Spotless heterozygote and with r^s a Ghost Spot heterozygote.

Other combinations, such as red leaf without spot, similar to the New World red (Harland, 1929), or red flower without spot, should never occur.

Harland's (1929) anthocyanin series in New World cottons can be brought into line by postulating separate gene centres for spot and red.

(5) *The bar allelomorph series in Drosophila.*

Sturtevant (1925 and 1928) studied mutation at the bar locus in *Drosophila*, and concluded that reversion of the bar gene to wild type resulted from the omission of the bar gene from between forked and fused by unequal crossing-over. This unequal crossing-over also resulted in the appearance of ultrabar—or double bar—by the insertion of the bar gene lost by one chromosome alongside the bar gene already present in the other chromosome. He supported his argument by studies on bar-infrabar. Infrabar and double infrabar are exactly analogous to bar and ultrabar. Infrabar arose from bar by a change in the bar gene and double

infrabar arose from it. Sturtevant was able to obtain two types of bar-infrabar, in which both a bar and an infrabar "gene" were present in the same chromosome. They mutated either to bar or to infrabar but not to full. Sturtevant was able to show that in "bar-infrabar," bar mutants usually arose in association with crossing-over between bar and fused, and infrabar mutants usually arose in association with crossing-over between bar and forked. In "infrabar-bar," the reverse was the case. Sturtevant argues that in bar-infrabar the order of the "genes" is forked, bar, infrabar, fused; and in infrabar-bar, it is forked, infrabar, bar, fused. From Sturtevant's results with bar-infrabar Thompson (1931) concluded that bar-infrabar carries one bar and one infrabar episome separately attached near the right and left poles of the protosome. Then if the bar episome is near the fused pole, infrabar mutants will be associated with crossing-over between fused and bar, and if the infrabar episome is near the fused pole, bar mutants will be associated with crossing-over between fused and bar.

Thompson's theory of the organisation of the bar gene may be brought into line with the present hypothesis by supposing that there are two gene centres in the bar protosome.

Sturtevant's data on the association of crossing-over with mutation in bar-infrabar is the best evidence available for two gene centres. On the present hypothesis, however, there should be two kinds of each of the genes, bar, ultrabar, infrabar, and double infrabar, distinguishable by their relative rates of mutation in forked and fused cross-over classes. When only one gene centre is occupied, it may be expected that the relation between crossing-over and mutation will not be so exact as when both are occupied, since in the former case there is the possibility of migration of the episome from one gene centre to the other, giving rise to genes which would mutate in association with forked-bar cross-overs where mutation associated with fused-bar cross-overs were expected, or *vice versa*. For the same reason the hypothesis can only be tested in strains in which the bar genes used have all arisen from a single bar gene within the last few generations.

A study of Sturtevant's (1925) paper revealed only one case in which it is certain that the above requirements were met. In his Table VIII he gives data for crossing-over and mutation in his original infrabar strain. Of 21 mutants, 17 were associated with infrabar-forked crossing-over, and only four with infrabar-fused crossing-over. In the original infrabar strain, therefore, the infrabar episome was attached to the gene centre nearest forked, and not at the mid-point of the protosome, as postulated by Thompson.

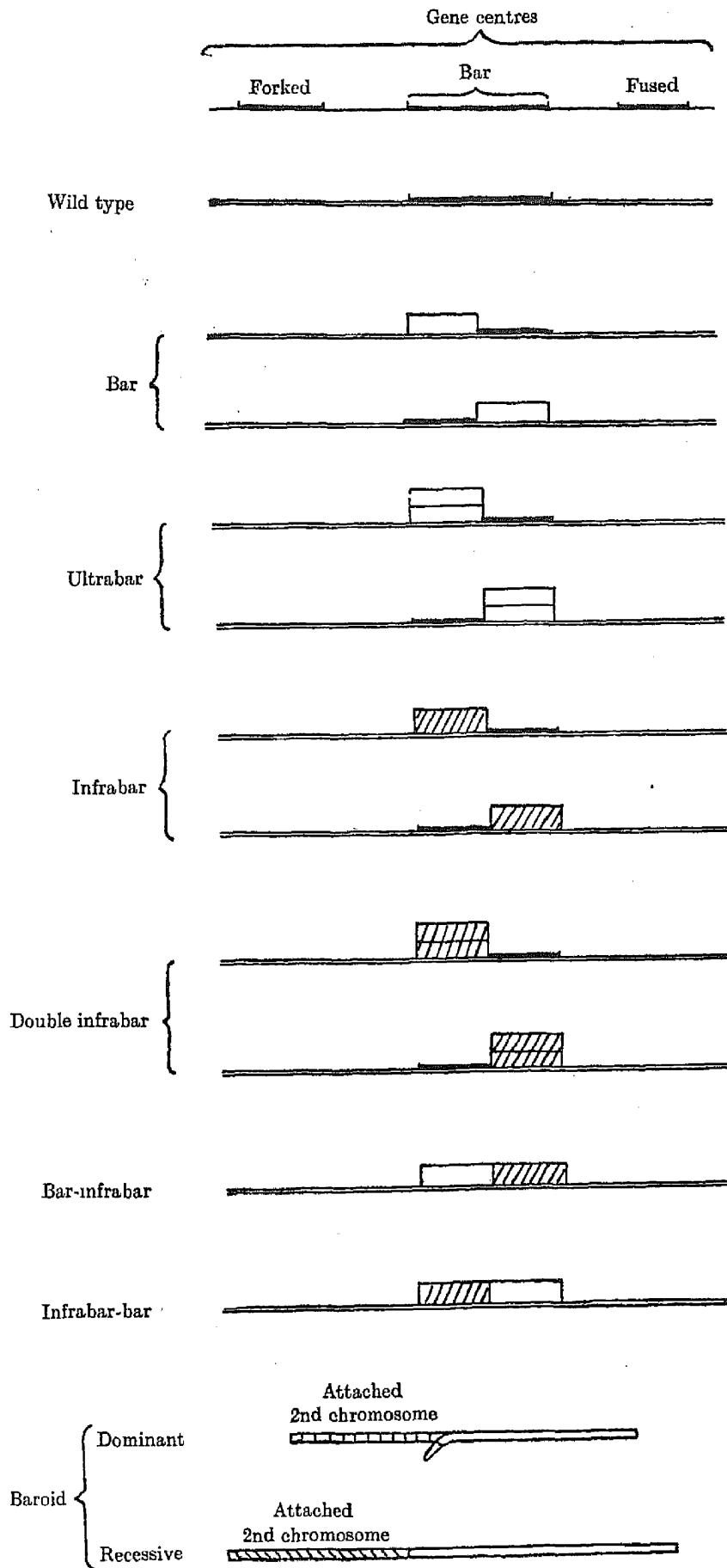
Suitable tests should separate the genes of the bar and infrabar series into two groups, those in which mutation is usually associated with bar-forked crossing-over, and those in which mutation is usually associated with bar-fused crossing-over.

Dobzhansky's (1932) baroid mutant at the bar locus is capable of a fairly simple explanation on the gene centre and episome theory. It may be supposed that the attached section of the second chromosome is attached closely to the bar gene, since there is no crossing-over between baroid and the locus of breakage. The effects of baroid are largely positional effects, since they are due to the attachment of a new chain to a bar gene centre, in place of the usual X-chromosome chain. Such a positional effect may be expected to be recessive to wild type, since no episomes are involved. If, on the other hand, the second chromosome chain were attached sub-terminally to the second gene centre of the bar protosome, the terminal gene centre would be displaced to form a side branch similar to that formed by the attachment of an episome. Such a gene should behave like bar, and give an intermediate heterozygote instead of being recessive. The arrangement might be expected to be unstable, and to mutate to the terminal arrangement. Baroid was, in fact, dominant over normal at its first appearance, but became recessive in the next generation.

Diagrams of the suggested organisation of the allelomorphs are given on page 507.

(6) *The scute allelomorph series in Drosophila.*

Agol (1931) and Dubinin (1932) working with a series of X-ray induced mutants at the scute locus, concluded "that the gene is not, at least not always, something homogeneous, indivisible, but may be represented by a complex organisation, composed of separate districts joined with each other, each possessing its specific action." They introduced the conception of the "gene centre," here used in a slightly different sense. If the gene material is a continuous rod, rather than a series of discrete particles attached to a rod, it follows that a mutation may occur which overlaps what were previously considered to be distinct, but neighbouring genes. Scute², which affects the wings in a manner similar to the spoon gene, and scute³, which affects most of the parts affected by scute and by achaete, were interpreted as such overlapping mutants. On the present theory these mutants may be interpreted as resulting from the loss of varying numbers of episomes from a series of gene centres arranged in linear order in the protosome. Episomes tend to be lost in groups, and not



singly or at random. The mutation from L^BK to lk observed in cotton is analogous to the overlapping mutants of Agol and Dubinin, except that the gene centres involved are in protosomes so far apart as to allow of about 30 per cent. of crossing-over between them.

(7) *Reddish α in Drosophila.*

Demerec (1928) found that mutation in *Drosophila virilis* from reddish α to wild type tended to occur in association with crossing-over between reddish and scute. Scute is 0.6 unit away from the locus of reddish. No reversions occurred among cross-overs between reddish and sepia, which is 2.2 units away on the other side of reddish from scute. It may be noted that reddish α originated in a fly carrying a chromosome in which crossing-over had occurred not more than two generations previously between sepia and scute, which suggests that the disturbance which gave rise to reddish α was similar to the disturbances which subsequently caused it to mutate.

The yellow-reddish allelomorph series can be brought into line with the present hypothesis by assuming an organisation of the wild allelomorph similar to that of mutant broad (L^B) in the cotton leaf shape series. If wild type has four episomes arranged one on each of four gene centres, yellow may be supposed to result from the loss of one episome. With only three instead of four gene centres occupied, yellow is recessive to wild type.

In the genesis of the reddish α gene it is suggested that the wild allelomorph of yellow was upset by crossing-over, and became rearranged, in one case giving rise to a heterozygous fly, and in the other to occasional gametes, one of which gave rise to a single reddish male. The four episomes may be supposed to have been rearranged on two gene centres, three on the terminal gene centre nearest to scute, and one on the next. This arrangement is unstable in heterozygotes, and mutates to wild type in 47 per cent. of cross-overs between reddish and scute. Reddish α is recessive to yellow, as would be expected on the hypothesis since only two gene centres are now active.

Reddish α mutates also to stable reddish. That the arrangement of the episomes is different in stable reddish from the arrangement in mutable reddish α is shown by the fact that, whereas reddish α is stable when homozygous, and unstable in heterozygotes with wild type and yellow, it is also unstable in heterozygotes with stable reddish. That it remains nearer to the scute gene than to sepia is shown by the fact that most of the reversions in low reversion stocks were also cross-overs in the reddish-

scute region. That the episome number is the same as in mutable reddish is shown by the fact that stable reddish mutates occasionally to wild type, and not to yellow. That not more than two gene centres are occupied is shown by the fact that stable reddish is recessive to yellow. It may therefore be supposed that reddish becomes stable by migration of one episome to give two episomes on each of two gene centres.

Diagrams of the suggested organisation of the yellow-reddish allelomorph are given on page 510.

If the genes of this series are organised as suggested, other allelomorphs recessive to yellow are theoretically obtainable by elimination of episomes from reddish. It may be suggested that by irradiation of reddish α there should be a reasonable prospect of obtaining at least two more members of the series, with two and no episomes respectively, thus:

Sub reddish



Basal member

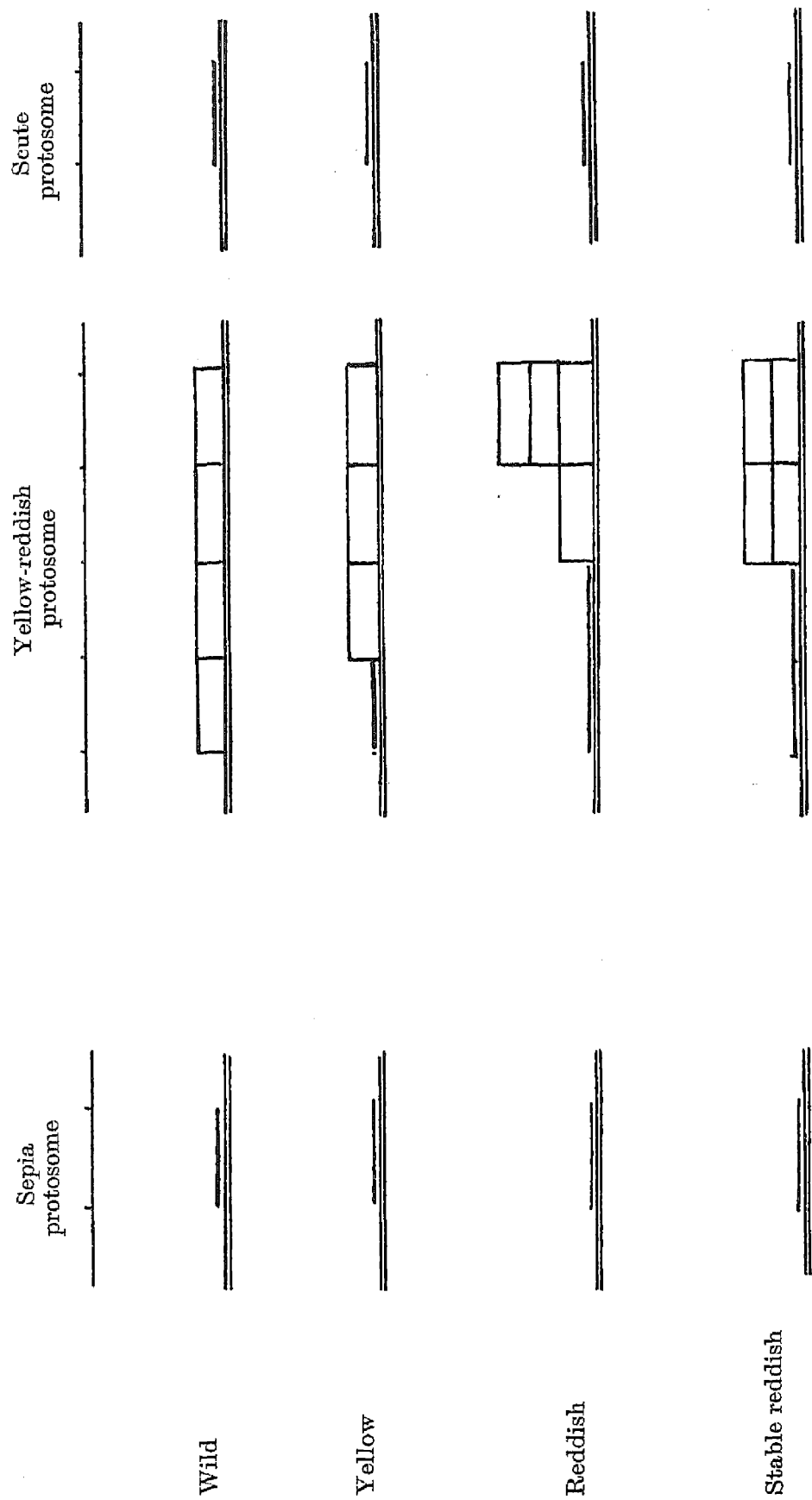


These may be expected to give intermediate heterozygotes with reddish, and to be completely recessive to yellow and wild type.

(8) "Rogue" in culinary peas.

The "rogue" factor in peas which was the subject of papers by Bateson and Pellew (1915, 1916, 1920) and Brotherton (1923, 1924) behaves in a manner similar to the leaf-shape mutants here reported. The gene for rogue designated X by Brotherton arises spontaneously by mutation from its allelomorph x and induces mutation in Xx heterozygotes just as L^B does in $L^B L^L$ heterozygotes, or as l does in $L^L l$ heterozygotes in the progeny of $764 \times$ Burma Laciniated. Brotherton was able to demonstrate single-factor segregations involving the rogue gene by crossing rogues with a non-rogue-producing strain. He postulates the existence of a relatively stable normal allelomorph x' . A good deal of mutation occurred even in his relatively stable Xx' heterozygotes, and his F_3 results suggest segregation of a factor or factors inducing mutability.

Brotherton assumes that X is dominant, but both he and Bateson and Pellew state that F_1 's between rogue and normal were normal looking in the young stages, and only those which remain somewhat intermediate during the early part of the flowering stage gave any normal or intermediate offspring. Rogues grown from rogue parents, on the other hand,



were rogue-like from the earliest stages. It is probable, therefore, that if a stable heterozygote could be obtained, it would be intermediate in character. This suggestion is borne out by the statement by Bateson and Pellew that intermediates with pointed leaflets gave predominantly rogues, and produced their normal offspring chiefly from the lower nodes, whereas intermediates with rounded leaflets—*i.e.* more nearly normal—produced predominantly normals, and produced their rogue offspring at random throughout the plant. If Xx heterozygotes are intermediate, the former type of intermediate may be regarded as an $Xx + XX$ mosaic which became an XX homozygote at maturity, and the latter type of intermediate as an Xx heterozygote which was relatively stable.

The occurrence of mosaics of tissue of different genotypes was first observed by Bateson and Pellew among their intermediates.

The unstable x , the relatively stable x' , and the mutant X are similar to the unstable *cernuum* L , the relatively stable *arboreum* L , and recessive broad derived by mutation from unstable types. The two former may differ only in factors affecting mutation rate, while the rogue gene X differs from the other two in lacking one episome.

(9) Dominance.

The theory of gene organisation throws light on the origin and nature of dominance. In the cotton leaf shape allelomorph series and the *Drosophila* yellow-reddish allelomorph series mutation to dominant allelomorphs takes place with considerable frequency. In these cases, where the relationships between the genes show that dominance is a function of the gene itself, it is ascribed to the activation of extra gene centres affecting the same character, by the redistribution of episomes. Where members of an allelomorph series differ in the number of episomes in a chain, the heterozygote is often intermediate, as in the lacination series in cotton leaf shape, and the bar and infrabar series in *Drosophila*. "Wild type" genes differing in episome number from their allelomorphs may become dominant over their allelomorphs by the accumulation of genes modifying the heterozygote, as shown by Fisher (1928*a*, 1928*b*, 1929) and Harland (1932*b*). From these cases there must be distinguished cases such as the anthocyanin series in Asiatic cotton (Hutchinson, 1932*b*), in which the allelomorphs differ in episome number, and the apparent dominance of genes of high episome number over those of lower episome number is due to the fact that the colour distribution is that of the higher member. The less intense colour of the heterozygote shows that it is actually intermediate in expression. In many cases in which domi-

nance has been reported, the heterozygote is intermediate, and "dominance" means no more than that the heterozygote is more easily separated from one homozygote than from the other. The reported dominance of *arboresum* L over *Nanking* l in cotton is a case in point, since it has been shown above that, although heterozygotes cannot be accurately distinguished from homozygous LL, they are in fact strictly intermediate between LL and ll.

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